Editorial Review

JUSTUS von LIEBIG

With the first number of the new volume the portrait of Lavoisier no longer graces the cover of the Journal; it has been replaced by that of Justus von Liebig, which one of his great-grandsons, Doctor Hesse, has permitted us to use for this purpose. The likeness has been prepared from a photograph of an oil painting by Trauschold which is still in the possession of the family.

Liebig was a chemist at a time when natural science and medicine had not yet broken loose from the fetters of speculation and philosophy. He introduced laboratory instruction, thereby giving to many for the first time a foundation for inventive experimentation. In consequence agricultural chemistry and physiology received a new guidance. Teaching concerning the nutrition of plants and animals owes to him a great deal in the way of new facts and of fruitful incentives by which we the living of to-day are often guided though quite unaware.

Liebig was born in 1803 at Darmstadt. His father was a dealer in dyestuffs. Many of these he himself prepared from directions in books on chemistry which he borrowed from the rich collection in the court library. Young Justus assisted him in the laboratory. This was, as he himself has written,[1] an excellent training for him. Here he laid the foundation for his art in experimentation; here he sharpened his powers of observation and his visual memory of many chemical processes. He made good use of his privileges in the court library.

[1] Ber. d. D. chem. Ges., 1890, Bd. 23, S. 817.

I read the books as they were placed there upon its shelves; from bottom to top, from right to left, it was all the same to me. I am certain that this manner of reading was of no special benefit to me in the acquisition of positive knowledge, but it did develop within me the incentive, which is proper to chemists even more than to other students of nature, of reflecting upon phenomena.

The profession of 'chemist' at that time did not exist. Therefore Liebig apprenticed to an apothecary, but was able to tolerate the situation for only a brief period. Thereafter he went to the university. But the chemistory taught there had nothing further to offer him. He went to Paris and worked with Gay-Lussac. There he became acquainted with Alex. von Humboldt, on whose warm recommendation he came in 1824 to Giessen as a second professor of chemistry, at first against the will of the faculty. It was therefore not made easy for him at the outset. But his ardor carried him through. He created the first teaching laboratory in Germany and thus made that small university for the time being the center of chemical study for the whole cultural world. His pupils became the most famous chemists of the time. His old laboratory still exists and is easily accessible, for Giessen is only an hour by railway from Frankfurt on the line to Berlin. No one should fail to visit this historic shrine and obtain for himself a living conception of the primitive apparatus with which Liebig and his pupils 100 years ago were able to obtain such beautiful results. Also his native city Darmstadt lies not far from Frankfurt, on the line to Basel, and possesses a Liebig museum well worth seeing.

In 1852 Liebig accepted a call to Munich. His health was impaired; consequently he relinquished the personal direction of the laboratory teaching. Authorship now engaged his time almost exclusively, and occupied the latter part of his life both richly and fruitfully. He died in 1873.

It would lead too far to picture all of Liebig's famous exploits in chemistry. Such a review also would be out of place here. But his accomplishments in physiology should be discussed and especially his influence on the doctrines of

nutrition. In this connection it is singular that none of the physiologists of that time was an immediate pupil of Liebig. Only a much later generation has recognized that chemistry is for the future one of its most indispensable aids, and that it cannot get along with physics and the microscope alone.

PLANT AND ANIMAL PROTEINS

We physiologists honor in Liebig the analyst who, with his own methods, first systematically investigated the organs of plants and animals as well as their feces, urine and bile. He established the protein content of legumes and cereals and called attention to the fact that they possess among themselves and with the albumins of the animal world almost the same elementary composition. In the course of this investigation he separated the nitrogenous from the non-nitrogenous organic foodstuffs and emphasized the fact that the same substances occur in our foods and as constituents of our bodies and that the latter arise from the former. The plastic (nitrogenous) foods together with water and minerals build up the body; from them proceed all phenomena of motion. The respiratory (non-nitrogenous) foods serve only for the production of heat. Liebig has often been misunderstood. Naturally he was also a child of his time and many of his expressions we should formulate somewhat differently and more clearly to-day. However, that does not gainsay the fact that Liebig was the first to envisage the relationship of foods correctly.

This conception of the differentness (Verschiedenheit) of the foodstuffs was both new and true and will endure, even in the light of newer conditions and relationships. Its simplicity and, one might say, its self evidence will never detract from the high honor of one who first expressed it, and indeed with entire clearness of knowledge of its significance.

These words of Bischoff uttered in 1874 in his memorable address on Liebig are as true to-day as they were at that time.

It was also Liebig, who in his famous and consequential Theory of Nutrition of Animals and Man, for the first time clearly worked out the simple circulation of the organic foodstuffs in nature. The first thought of this had come to him while in Paris. He himself writes concerning it:[2]

I recognized, or probably more correctly, it glimmered in my consciousness that not only was there a law of relationship governing all chemical phenomena in the mineral, vegetable and animal kingdoms, that none stands alone, but always is closely interlinked with another, and this one again with another, and so on, all bound together, but also that the origin and occurrence of things is like a wave motion moving in a circle.

LAW OF CONSERVATION OF ENERGY

With the help of the solar energy plants assimilate and make available their body substances to all other living beings for dissimilation. This view led Liebig in 1848,[3] therefore before J. R. Mayer, Hemholtz and Joule, to the law of the conservation of energy and its validity in the animal body. In his earliest deductions he considered it unnecessary to throw overboard a peculiar life force; but it was for him only a principle of orientation in the organized world and not a particular form of energy as it appeared to his contemporaries. In the later editions of his 'chemical letters' he expressed himself more and more clearly. And this, although the numerically exact proof for the validity of the law in the animal body was only to become available much later. We marvel at the fruitful clearness of his ideas, in which he was far in advance of his contemporaries.

FAT FORMATION FROM PROTEIN

It is only natural that his views concerning processes of intermediary metabolism should differ essentially from those which are held probable to-day. Liebig at first sought in the

[2] Ber. d. D. chem. Ges., 1890, Bd. 23, S. 824.

[3] The author must be in error regarding this date, as Mayer's contribution is dated 1842 and that of Helmholtz 1847.—Ed.

(plastic) proteins the mother substance for deposited fat; later he recognized that it must come in far greater part from starches. This appears to us now as a self-evident and well-established datum of physiology. At that time it was not so. It was still in Liebig's life time that Voit strove so earnestly to bring the earlier doctrine to victory, and fell into the well-known controversy with Pflüger. The latter denied the formation of fat from protein. Bischoff was not so positive; he came to his memorial address on Liebig in 1874[4] to a *non liquet*. "However the final answer to the question, still actively discussed, may turn out, it will always remain an extraordinarily great service of Liebig to have brought it under investigation." The conflict of opinions was at that time maintained with a liveliness which to-day is not rightly understood. For the arguments and counter arguments which were adduced concerning fatty degeneration of organs, formation of adipocere, fermentative processes, inadequate respiration experiments, we do not regard to-day as any of them tenable. Fundamentally new material concerning the actual possibility of fat formation from protein has been brought forward only recently. But the question is not yet solved. It will be done only when we get an insight into the actual intermediary processes taking place, i.e., as, when and to how great an extent, probably persistent reductions in the strict sense are coupled with cleavages and oxidative metabolism.

MINERALS, LAW OF MINIMUM

Liebig's works on the mineral constituents of the organism have become best known. They constitute the point of departure for the 'law of minimum,' the significance of which does not need to be discussed more intimately here. Even to-day almost every issue of this Journal brings several contributions based upon this law. Originally it was deduced by Liebig from the ash analysis of different plants. That mineral constituent, which in relation to the need of the fruit is present

[4] Bischoff: Liebig's Einfluss auf die Entwicklung der Physiologie. München Akademie.

in the soil in the smallest quantity limits the plant's growth and determines the harvest yield. To-day we know that this law holds in general in the nutrition of every human being, and not for the minerals only, but for all constituents of the food, which cannot be prepared from other constituents. It holds for individual amino acid and led to the discovery of the vitamins; but it applies indeed also for definite kinds of sugar and fatty acids. It has occasioned one of the greatest branches of industry, the manufacture of artificial fertilizers, has led to the establishment of agricultural experiment stations, and, finally, it dominates even the commercial relations of foreign peoples one with another.

ACID-BASE ECONOMY OF SOILS AND OF LIVING BEINGS

Liebig found in the mineral constituents of the food the cause of the acid or alkaline reaction of the urine. The soils also show different reactions. One must seek plants adapted to a given soil. Its properties are changed in that definite minerals are withdrawn by the crops. Hence the necessity of rotation of crops and of fallow ploughing. From the views first promulgated and correctly developed with intuitive insight by Liebig have grown up the great doctrines of acid-base economy in both the living and the non-living worlds.

DIFFUSION

Liebig sought to find a basis for the differences in ash content of individual organs and thus came to a conception of disequilibria which are maintained in the life processes, and thence to the laws of diffusion. All this has for a long time become so generally accepted that we never reflect that all these conceptions are not yet 100 years old, all trace back to Liebig, and must have been won only by sagaciously devised experiments and by immensely painstaking analyses. Who knows to-day, for example, that it was Liebig who first made clear the detergent action of many neutral salts?

SOIL COLLOIDS

It is well known that the practical application of mineral fertilization was brought about by Liebig only after many years of the greatest disappointments and the severest hostility on the part of agriculture. For in the thought that the plants can take up only the dissolved minerals of the soil water and their impoverishment must be prevented, he with great pains transformed the phosphates into an insoluble form. Later he learned to recognize the great significance of the soil colloids and their powers of adsorption. I believe we as successors cannot be thankful enough that Liebig, through his obsession and his belief in the ideas of his time, was compelled to make this detour. Only by means of this has agricultural chemistry attained a secure foundation. And I am convinced that we animal physiologists shall still derive much of usefulness from these investigations. For *on* the colloid protoplasm, not in the tissue water, is where all intermediary processes take place. The identical laws, according to which the root hairs separate the soluble soil minerals from their adsorption compounds with the soil colloids and thus make them absorbable, are valid also for all those processes by which dissolved foodstuffs are taken up by the protoplasm of the cell, are directed to the proper places and then are transformed in an orderly but compelling fashion. Only thus can the life processes of the cell be maintained in an orderly progress.

FERMENTATION, CATALYSIS

In this field also, as it happens, Liebig not only advanced certain ideas but himself contributed very important research material in great abundance. His fundamental investigations on fermentation have become well known through his controversy with Berzelius and with Pasteur. They were not able, however, to lead him to an actual theory of catalysis. That could only come, as the history of every science shows, when quantitative measurements of the detailed processes which play a role in the 'drama' (Schönlein) of a catalytic reaction

are at hand. This became possible only after 1900 in an age in which the major industries were already making use of catalytic reactions. What a contrast between that day and this! Scarcely 100 years ago Liebig's small laboratory and his investigations on the nitrogen metabolism of plants, mineral fertilization, the fermentation of yeast; to-day the most imposing factories in which gas reactions are carried out with heterogeneous catalysis on the largest scale and ammonia and saltpeter become available to agriculture. Industry has extended Liebig's ideas and brought them to undreamed of fluorescence.

MEAT EXTRACTS

In an even wider field Liebig's discoveries and ideas are still to-day bringing forth fruit. His famous investigations on the composition of meat and his extractive substances have taught us to know creatin, creatinine and inosinic acid. Liebig was unable to gain a correct view of the physiological significence of these substances. Bischoff writes in this connection:[5]

> Now it is actually possible to assume that none of these extractive substances are constituents of the living organs in the form in which we obtain them by chemical treatment of the organs. And so it seems to me the more remarkable that Liebig was correct in his opinion concerning the substances effective in the muscle in relation to its activity, since everything which has been concluded to the contrary from the experimentally demonstrated ineffectiveness of them (for example creatin) has fallen down.

Is this not proof of a magnificent foresight that a half century later the precursors of these compounds, creatine-phosphoric acid and adenylic acid, have come to be known and now for the first time we are beginning to get an insight into the role which those substances play in the mechanism of muscular activity.

This review has already become too long, for in the richness of significant discoveries which we owe to Liebig it is difficult to make the right choice. I believe, however, it has been

[5] Loc cit., p. 84.

shown that Liebig's portrait has been rightly chosen to appear on the cover page of this Journal in the immediate future. Many of his ideas concerning the interdependence of events in metabolism appear to us no longer convincing; that in the progress of our knowledge is not at all surprising. Many of his experimental findings indeed are no longer valid at all; that also is not to be wondered at. For the correctness of experimental findings depends absolutely and all together on the mode of procedure by which they are obtained, and in methodology very naturally we have made much progress in the decades which have elapsed since Liebig's death. This is true especially of his physiological studies and Liebig himself was perfectly aware that this would be the case.

It is a fundamental postulate for researches in physics and in chemistry, the inorganic sciences, never to leave the path from the known to the unknown and to proceed from the study of simple phenomena to those which are more complex. It is not always possible to proceed in this manner in the investigation of the life processes. What is there in this field that is known with equal certainty? Imagination is allowed a much wider range. Careful to the utmost degree in his analyses Liebig nevertheless possessed imagination and therefore he was able in the latter half of his life to make the applied sciences productive in so astonishing a manner which prevails even to this day. To these applied sciences belongs the science of nutrition.

I ascribe thus to Liebig a very great and very beneficent influence on the development of a better and more exact method of investigation in physiology and in medicine. And I stress these deserts of Liebig all the more because the effects of their influence will extend far beyond his own individual accomplishments. Yet it is just these influences which the present generation, and still more the future generations, will forget all too early to ascribe to him. Particularly those who grew up instructed in these better methods of investigation are very much inclined to think that conditions always have been as good. Besides, only a few have a liking for historical studies. The majority of people scarcely know anything about

the operations of science beyond the juncture of their own consciousness.[6]

This review ventures to do justice in this respect to the memory of Liebig.

KARL THOMAS.

Physiological Chemical Institute,
University of Leipzig,
Germany.

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[6] Bischoff, loc. cit., p. 61.

THE BIOLOGICAL VALUE OF RATIONS CONTAINING FISH MEAL[1]

J. L. St. JOHN, J. S. CARVER, OTTO JOHNSON, S. A. MOORE AND HAROLD GERRITZ

Divisions of Chemistry and Poultry, Agricultural Experiment Station, Pullman, Washington

ONE FIGURE

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This paper presents results showing the effect of adding varying amounts of protein in the form of Alaska herring fish meal to a basal ration fed to growing chicks. St. John, Carver, Helphrey, Miller and Cassel ('30) reported results obtained with dry skim milk as a protein supplement, but were unable to calculate the biological value of the protein of those rations, because a satisfactory method for determining the biological value of protein suitable for use with poultry was not available. St. John, Johnson, Carver and Moore ('32) designed a method for this purpose, and biological values for nitrogen are reported in the present paper. It would be highly desirable to use a so-called synthetic ration composed of purified food materials for this type of work, but, as shown in this laboratory (Helphrey, '29; Aspinall, '30) and by the work of others, such a ration has not been successfully used with poultry.

The general theory upon which the determination of biological value is based is discussed by Thomas ('09), Mitchell ('24, '26), Mitchell and Hamilton ('29) and others, and the reader is referred to these papers for this material.

[1] Published as scientific paper no. 274, College of Agriculture and Agricultural Experiment Station, State College of Washington.

EXPERIMENTAL

Four lots of S. C. White Leghorn chicks were used, each lot consisting of thirty chicks which were placed in the nutritional batteries at the age of 48 hours. Each chick was weighed at the beginning of the experiment and once each week thereafter. A record of feed consumption and of the amount of excreta for each lot was kept and is reported for weekly periods. The method of caring for the chicks is described by St. John, Carver, Helphrey, Miller and Cassel ('30). The experiments were continued for 12 weeks beginning January 27, 1931. The excreta were collected, preserved and analyzed according to the methods described by St. John and Johnson ('31)[2] and St. John, Johnson, Carver and Moore ('32), except that, with the type of ration used in these experiments, it was found desirable to use 0.65 N hydrochloric acid for the preservation of the excreta. Swift, Black, Voris and Funk ('31) have recently emphasized the need for improved methods of experimental procedure in poultry work. Using the methods suggested by St. John and Johnson ('31) it has been possible to prepare a uniform sample for the determination of the uric acid, to make a quantitative determination of the uric acid, and to remove the feathers from the excreta after treating with hydrochloric acid and drying and before pulverizing. However, the number of feathers lost in the pens was reduced to a minimum by the method developed by Carver ('31). With the batteries and feed hoppers used (St. John, Carver, et al., '30, and Carver, St. John, et al., '32) there was practically no mixing of food and excreta.

The basal ration used consisted of: Ground yellow corn, 45 pounds; ground wheat, 25 pounds; wheat bran, 20 pounds; alfalfa leaf meal, 5 pounds; ground oyster shell, 3 pounds; bone meal, 1 pound; salt, 1 pound; fortified cod liver oil, ¼ pound. No scratch grain was fed at any time, the all-mash ration being fed exclusively. To this the Alaska herring meal was added to give the ratios shown in table 1. The per cent

[2] In this method, the paper used for filtering the alkaline solution after adding piperidine and heating was S & S no. 588.

of nitrogen in the fish meal was 10.66 (N $\times$ 6.25 equals 66.6 per cent protein). The proximate analysis of the rations is shown in table 1.

TABLE 1
Composition of rations

LOT	RATIO BASAL RATION TO FISH MEAL	MOISTURE	ASH	ETHER EXTRACT	CRUDE FIBER	NITROGEN	PROTEIN
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
55	97: 3	11.2	5.8	4.9	5.3	2.10	13.1
56	93: 7	10.7	6.2	4.1	4.9	2.48	15.5
57	88: 12	10.8	6.3	4.0	4.8	3.10	19.4
58	80: 20	10.6	6.4	3.6	4.7	3.89	24.3

The growth curves are shown in figure 1, based upon the data shown in table 2.

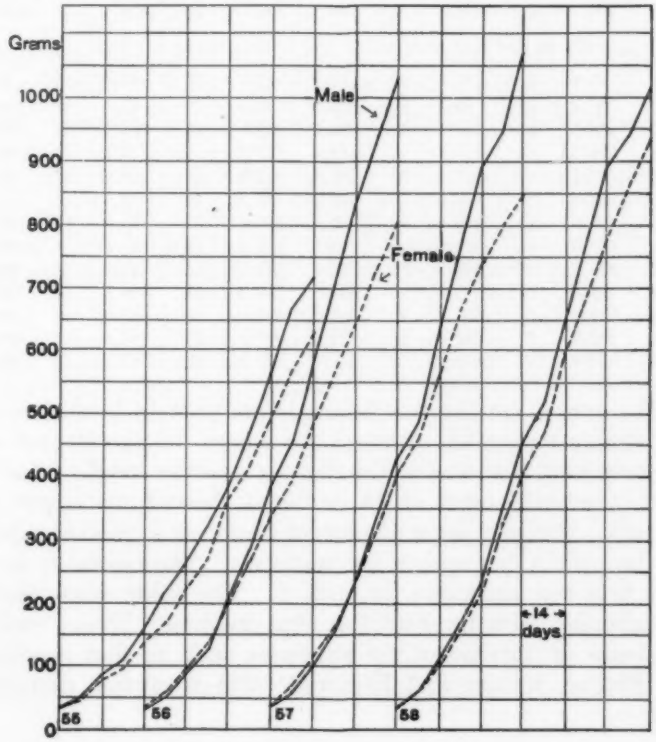

TABLE 2

Average weight of chicks by weeks

WEEKS	GM.	MALE		FEMALE		Gm.	MALE		FEMALE	
		No.	Gm.	No.	Gm.		No.	Gm.	No.	GM.
	Lot 55—13 per cent protein					Lot 56—15 per cent protein				
0	37.6	17	38.2	11	37.2	36.1	12	35.5	13	36.4
1	50.1	17	51.3	11	49.2	52.5	12	50.8	13	53.8
2	83.8	17	86.5	11	79.7	91.3	12	87.5	13	94.9
3	105.3	17	109.9	11	98.2	131.2	12	127.5	13	134.6
4	152.0	17	159.8	11	140.2	200.8	12	202.7	13	199.2
5	197.5	16	213.1	9	169.8	277.5	12	287.7	13	268.2
6	251.4	16	266.3	9	224.9	360.4	12	382.7	13	340.0
7	303.2	16	319.8	8	270.0	421.4	12	455.0	13	390.5
8	378.2	16	384.1	8	366.4	528.2	11	581.5	13	483.1
9	452.8	16	468.4	8	421.8	628.6	11	704.7	13	564.2
10	541.7	16	564.4	8	496.5	734.3	11	836.4	13	647.9
11	634.3	16	666.8	8	569.5	830.7	11	945.6	13	733.7
12	690.0	16	719.6	8	630.8	910.0	11	1034.7	13	804.6
	Lot 57—19 per cent protein					Lot 58—24 per cent protein				
0	37.7	17	37.7	13	37.7	38.4	16	38.8	13	38.2
1	56.3	17	54.9	13	58.2	56.3	16	57.0	13	57.0
2	104.4	17	100.6	13	109.5	109.0	16	111.7	13	105.8
3	156.3	17	153.1	13	160.6	170.7	16	173.3	13	167.5
4	234.7	16	234.4	13	235.3	232.5	16	238.3	13	225.6
5	329.0	16	337.3	13	318.9	341.3	16	352.1	13	328.2
6	419.8	16	429.8	13	407.7	432.6	16	452.0	13	408.9
7	474.3	16	489.3	13	456.0	505.4	16	527.5	13	478.2
8	608.1	15	646.9	13	563.4	629.4	14	656.0	13	600.9
9	728.9	15	787.0	13	661.8	739.4	14	780.3	13	695.4
10	820.4	15	891.2	13	738.8	837.6	14	889.1	13	780.0
11	907.0	15	999.1	13	800.1	907.8	14	942.4	13	870.5
12	966.3	15	1070.3	13	846.3	978.3	13	1017.7	13	938.9

The curves for the two intermediate protein levels are approximately parallel, although the chicks fed the 19 per cent protein level had attained a slightly greater weight. Lot 55 with a protein level of 13 per cent showed distinctly less growth. The high level of protein produced a greater growth in the pullets, but less in the cockerels. The mortality in all lots was low as shown in table 2. The birds were thrifty throughout, with no disabling abnormalities. There was no evidence of nutritional leg paralysis such as that described by Bethke, Record and Kennard ('31) when fish meal was fed.

The average feed consumed per bird per week is shown in table 3.

TABLE 3

Average feed consumed per bird per week (grams)

WEEK	LOT 55	LOT 56	LOT 57	LOT 58
1	41.1	40.7	41.7	41.4
2	87.0	95.6	99.0	96.1
3	100.9	113.6	130.6	126.8
4	149.5	186.4	184.8	192.3
5	196.1	224.3	232.2	242.3
6	211.9	274.3	307.7	276.7
7	246.9	277.4	285.8	318.7
8	265.6	337.1	327.6	344.0
9	391.3	433.3	364.3	429.9
10	365.3	353.0	365.6	400.0
11	415.5	443.2	430.7	436.5
12	466.4	508.2	444.2	451.5

The only consistent variation in feed consumption between the four lots is found in the lower consumption by lot 55, which received the ration containing the lowest percentage of protein.

In table 4 is shown the amount of gain per unit of feed consumed by the chicks. In general the gain per unit of feed is somewhat larger for lots 56, 57, and 58 than for lot 55. This continues through the tenth week, but is not true for

TABLE 4

Grams gain per gram of feed

WEEK	LOT 55	LOT 56	LOT 57	LOT 58
1	0.27	0.34	0.44	0.43
2	0.37	0.34	0.49	0.53
3	0.21	0.35	0.40	0.49
4	0.31	0.37	0.40	0.32
5	0.14	0.36	0.41	0.45
6	0.25	0.29	0.30	0.33
7	0.17	0.22	0.19	0.23
8	0.28	0.26	0.36	0.25
9	0.19	0.23	0.33	0.26
10	0.24	0.30	0.25	0.24
11	0.22	0.22	0.20	0.16
12	0.12	0.16	0.13	0.08

each weekly period up to this age. For the eleventh and twelfth weeks the efficiency of the feed in producing growth decreases noticeably. Table 5, showing the gain per unit of

TABLE 5

Grams gain per gram of protein

WEEK	LOT 55	LOT 56	LOT 57	LOT 58
1	2.07	2.18	2.29	1.78
2	2.79	2.20	2.51	2.17
3	1.62	2.26	2.05	2.00
4	2.38	2.41	2.04	1.32
5	1.06	2.32	2.09	1.85
6	1.94	1.86	1.52	1.36
7	1.28	1.42	0.98	0.94
8	2.15	1.71	1.84	1.04
9	1.45	1.50	1.71	1.05
10	1.86	1.93	1.29	1.00
11	1.70	1.40	1.04	0.69
12	0.91	1.01	0.69	0.32

protein consumed, gives more definite information regarding the relative efficiency of different levels of protein. During the entire experimental period lot 56, receiving a ration containing 15 per cent protein, showed, on the average, the most efficient use of the total protein in the ration, while the low protein lot 55 was second. The high protein lot 58 was decidedly less efficient in its use of protein. During the first half of the period, lot 57 was somewhat more efficient than 55, but during the second half of the period the reverse was true; and in fact this latter was the most efficient of the four during this second half. The efficiency of protein utilization decreases with age, as was previously noted by St. John, Carver, Helphrey, Miller and Cassel ('30). This decrease is much more marked in the high protein lot 58 than in the other lots.

In tables 6 to 9 are given the data necessary for the calculation of the biological value of nitrogen. Detailed information on nitrogen intake and excretion are presented.

TABLE 6

Nitrogen intake and excretion at 13 per cent protein level (lot 55)

WEEKS	TOTAL DRY MATTER	URIC ACID	TOTAL NITROGEN	AMMONIA NITROGEN	URINARY NITROGEN	FECAL NITROGEN	TOTAL WEIGHT OF CHICKS	TOTAL FEED CONSUMED	TOTAL NITROGEN IN FEED CONSUMED	METABOLIC NITROGEN	ENDOGENOUS NITROGEN	BIOLOGICAL VALUE
	Gm.	*Per cent*	*Per cent*	*Per cent*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
0							1130					
1	340.2	5.11	3.92	0.216	8.163	5.173	1454	1192	25.03	3.397	4.122	82.62
2	696.1	2.94	3.33	0.600	13.749	9.431	2347	2436	51.16	6.943	6.654	85.22
3	966.8	3.14	3.26	0.540	19.175	12.343	2948	2825	59.33	8.051	8.358	80.35
4	1478.6	4.43	3.20	0.124	29.585	17.730	4258	4188	87.95	11.936	12.071	78.68
5	1544.8	4.87	3.24	0.136	33.973	16.079	4938	4904	102.98	13.976	13.999	80.20
6	1744.4	3.71	3.42	0.440	36.559	23.099	6284	5299	111.28	15.102	17.815	81.85
7	1820.7	3.67	3.81	0.636	42.316	27.053	7276	5926	124.45	16.889	20.627	81.02
8	2030.0	1.57	3.54	1.104	41.294	30.568	9077	6375	133.88	18.169	25.733	87.19
9	2905.7	2.97	3.14	0.520	54.845	36.394	10868	9391	187.82	26.764	30.811	86.51
10	3203.5	2.26	3.14	0.640	55.794	44.796	13002	8768	175.36	24.989	36.861	87.83
11	3094.7	2.07	3.66	0.982	64.679	48.587	15224	9972	199.44	28.420	43.160	88.00
12	3256.5	2.53	3.90	1.092	78.780	48.224	16558	11193	223.86	31.900	46.942	84.66

TABLE 7

Nitrogen intake and excretion at 15 per cent protein level (lot 56)

WEEKS	TOTAL DRY MATTER	URIC ACID	TOTAL NITROGEN	AMMONIA NITROGEN	URINARY NITROGEN	FECAL NITROGEN	TOTAL WEIGHT OF CHICKS	TOTAL FEED CONSUMED	TOTAL NITROGEN IN FEED CONSUMED	METABOLIC NITROGEN	ENDOGENOUS NITROGEN	BIOLOGICAL VALUE
	Gm.	*Per cent*	*Per cent*	*Per cent*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
0							1085					
1	343.7	4.78	4.14	0.216	7.773	6.456	1470	1142	28.32	3.255	4.167	85.64
2	671.1	3.25	3.73	0.500	13.283	11.749	2284	2391	59.30	6.814	6.475	87.48
3	987.8	4.68	3.74	0.240	22.226	14.718	3280	2840	70.43	8.094	9.299	79.74
4	1535.9	4.45	3.54	0.136	31.089	23.282	5021	4661	115.59	13.284	14.235	84.04
5	1860.4	4.87	3.54	0.208	42.588	23.270	7038	5608	139.08	15.983	19.952	82.82
6	2092.0	3.76	3.92	0.688	50.766	31.240	9012	6854	169.98	19.534	25.549	84.07
7	2103.8	3.43	4.26	1.036	57.310	32.312	10536	6934	171.96	19.762	29.870	82.79
8	2568.2	1.93	3.90	1.232	60.203	39.957	12676	8090	200.63	23.056	35.936	86.79
9	3119.6	3.94	3.52	0.480	69.931	39.879	15087	10400	239.20	29.640	42.772	88.14
10	3321.0	2.69	3.75	1.056	81.060	43.478	17623	8472	194.86	24.145	49.961	82.28
11	3046.8	1.93	4.18	1.540	83.153	44.203	19939	10638	244.67	30.318	56.527	88.46
12	3471.9	3.10	4.50	1.464	109.631	46.605	21842	12196	280.51	34.759	61.922	82.24

TABLE 8

Nitrogen intake and excretion at 19 per cent protein level (lot 57)

WEEKS	TOTAL DRY MATTER	URIC ACID	TOTAL NITROGEN	AMMONIA NITROGEN	URINARY NITROGEN	FECAL NITROGEN	TOTAL WEIGHT OF CHICKS	TOTAL FEED CONSUMED	TOTAL NITROGEN IN FEED CONSUMED	METABOLIC NITROGEN	ENDOGENOUS NITROGEN	BIOLOGICAL VALUE
	Gm.	*Per cent*	*Per cent*	*Per cent*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
0							1132					
1	386.6	5.11	4.40	0.256	9.469	7.541	1689	1253	38.84	3.571	4.788	86.58
2	910.7	5.26	4.46	0.420	24.741	15.876	3134	2970	92.07	8.465	8.885	81.27
3	1386.7	6.33	4.22	0.192	39.901	18.618	4690	3919	121.49	11.169	13.296	76.67
4	1888.7	6.17	4.46	0.239	54.198	30.038	6809	5360	166.16	15.276	19.304	76.95
5	2457.8	6.76	4.74	0.376	80.779	35.721	9542	6735	208.79	19.195	27.052	72.06
6	2565.2	5.73	5.12	0.940	91.385	39.953	12176	8923	276.61	25.431	34.519	78.30
7	2679.5	6.06	5.46	1.016	101.688	44.613	13756	8289	256.96	23.624	38.998	73.43
8	2919.8	3.66	5.10	1.672	105.550	43.360	17028	9174	284.40	26.146	48.274	78.56
9	3690.0	6.42	4.64	0.496	121.585	49.631	20410	10202	299.94	29.076	57.862	77.19
10	3906.7	4.12	4.85	1.352	133.089	56.386	22972	10238	301.00	29.178	65.126	75.18
11	3434.5	3.73	5.29	1.912	135.463	46.222	25396	12061	354.60	34.374	71.998	81.48
12	3686.8	4.41	5.42	1.744	148.118	51.707	27056	12438	365.68	35.448	76.704	79.56

TABLE 9

Nitrogen intake and exoretion at 24 per cent protein level (lot 58)

WEEKS	TOTAL DRY MATTER	URIC ACID	TOTAL NITROGEN	AMMONIA NITROGEN	URINARY NITROGEN	FECAL NITROGEN	TOTAL WEIGHT OF CHICKS	TOTAL FEED CONSUMED	TOTAL NITROGEN IS FEED CONSUMED	METABOLIC NITROGEN	ENDOGENOUS NITROGEN	BIOLOGICAL VALUE
	Gm.	*Per cent*	*Per cent*	*Per cent*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	
0							1152					
1	364.1	7.15	5.66	0.288	12.159	8.449	1689	1243	48.35	3.543	4.788	83.03
2	838.9	8.58	5.98	0.448	34.689	15.477	3163	2789	108.50	7.949	8.967	74.53
3	1269.9	9.25	5.82	0.272	53.261	20.647	4950	3677	143.04	10.479	14.033	70.48
4	1950.1	9.40	5.89	0.272	83.009	31.852	6745	5578	216.98	15.897	19.122	68.22
5	2202.2	9.95	6.29	0.448	103.633	34.885	9899	7027	273.35	20.027	28.064	70.77
6	2719.4	9.63	6.55	0.788	135.903	42.218	12548	8027	312.25	22.877	35.574	65.75
7	2877.0	10.91	7.03	0.992	166.459	35.749	14656	9244	359.60	26.345	41.550	64.33
8	2941.9	5.46	6.60	2.416	155.774	38.391	16996	9290	361.38	26.477	48.184	69.21
9	3646.9	10.10	6.23	0.808	190.308	36.894	19964	11608	427.18	33.083	56.598	68.42
10	4018.7	9.24	6.73	1.460	228.061	41.985	22588	10802	397.52	30.786	64.037	57.54
11	3409.4	8.74	7.46	2.380	225.589	28.752	24510	11786	433.73	33.590	69.486	64.41
12	3587.0	9.59	7.31	1.976	231.929	30.281	25436	11740	432.04	33.459	72.111	63.28

The amount of dry matter excreted increases as the per cent of nitrogen in the ration increases up to the highest level where there is a decrease. There is also a marked increase in the per cent of total nitrogen in the excreta as the level of nitrogen in the feed increases. In fact, this amount of nitrogen in the excreta of birds receiving a feed containing 24 per cent protein is from one and one-half to two times as much as in the excreta of those receiving only 13 per cent protein in the feed. The per cent of uric acid in the excreta is approximately the same for lots 55 and 56, but is higher for lot 57, and is markedly higher for lot 58. The per cent of uric acid in the excreta of the latter lot is in most cases from two to four times that in lot 55, the low protein lot. The per cent of ammonia nitrogen fluctuates, but shows little consistent variation with protein levels, except in the latter part of the experimental period, when it is somewhat higher in the higher protein lots. The per cent of ammonia nitrogen in the excreta using these rations was materially higher than that found by St. John, Johnson, Carver and Moore ('32) in the excreta when a low nitrogen diet was fed.

It appears that the urinary nitrogen may be expected to increase with an increase in the protein level in the feed. Inspection of tables 6 to 9 shows that in the early part of the experimental period the amount of urinary nitrogen eliminated by lot 58 is approximately two and one-half times as great as the amount eliminated by lot 55. The difference increases, until at the tenth week there is four times as much eliminated by the high as by the low protein lot. At this point lot 58 seems to have reached a maximum, while the other three lots continue to increase throughout the 12-week period. Through the 12 weeks lots 56 and 57 are intermediate between the other two, showing a progressive increase from low to high protein feeding levels.

The data for fecal nitrogen show a variation quite different from the urinary nitrogen. There is some increase in the fecal nitrogen with increased nitrogen feeding level during the first half of the experimental period. During the latter

half of the period the fecal nitrogen is less in lot 58. During the last 2 weeks it is approximately the same in the first three lots. It may also be noted here that the amount of urinary nitrogen is materially higher than the fecal nitrogen. The same was found by St. John, Johnson, Carver and Moore ('32) using a low nitrogen diet.

From the data recorded in tables 6 to 9, the biological value of the rations containing varying levels of protein were calculated each week by the method proposed by St. John, Johnson, Carver and Moore ('32). A summary of these values is presented in table 10. The biological values are based on group averages. Such a procedure was recommended by Ackerson, Blish and Mussehl ('23) as a result of the study of individual variation. Swift, Black, Voris and Funk ('31), in a preliminary paper, believe that individual birds in separate cages should be used. Ackerson, Blish and Mussehl ('30) present evidence to support their contention that biological values should be based on group averages.

TABLE 10

The biological values of rations containing Alaska herring fish meal at various nitrogen levels

WEEKLY PERIOD	LOT 55 13 PER CENT PROTEIN		LOT 56 15 PER CENT PROTEIN		LOT 57 19 PER CENT PROTEIN		LOT 58 24 PER CENT PROTEIN	
	A[1]	B[2]	A	B	A	B	A	B
1	82.62	81.30	85.64	84.73	86.58	85.92	83.03	82.41
2	85.22	84.04	87.48	86.42	81.27	80.12	75.53	73.40
3	80.35	78.58	79.74	78.26	76.67	75.43	70.48	69.44
4	78.68	76.71	84.04	82.84	76.95	75.78	68.22	66.89
5	80.20	78.44	82.82	81.77	72.06	70.81	70.77	69.77
6	81.85	80.36	84.07	83.12	78.30	77.32	65.75	64.76
7	81.02	79.48	82.79	81.94	73.43	72.57	64.33	63.36
8	87.19	86.34	86.79	86.18	78.56	78.03	69.21	68.59
9	86.51	85.33	88.14	87.45	77.19	76.75	68.42	67.68
10	87.83	87.02	82.28	81.91	75.18	75.00	57.54	57.02
11	88.00	87.24	88.46	88.11	81.48	81.25	64.41	64.03
12	84.66	83.69	82.24	81.61	79.56	79.38	63.28	62.95
Average	83.68	82.38	84.54	83.70	78.10	77.36	68.33	67.52

[1] Based on metabolic nitrogen per kilogram of feed.

[2] Based on metabolic nitrogen per kilogram of body weight.

Column A in table 10 gives the biological values calculated on the basis of a constant metabolic nitrogen value per kilogram of feed consumed. The biological values based on the metabolic nitrogen per kilogram of body weight are also given in column B. It is apparent that the values in column A are higher, although the difference between the two is slight. It is apparent that the biological value is higher for lots 55 and 56. This is especially evident if these data are plotted. This is apparent both from a comparison of the values week by week and from the averages. Lots 57 and 58 show an appreciably lower biological value. It should also be noted that lot 58, which received the ration containing the highest per cent of protein, shows a decrease in biological value with increasing age of the chicks. There is variation from week to week in all lots, but a general decrease with advancing age in lot 58. The level of the biological value remains about the same throughout the experimental period for the other three lots.

Preliminary to the work just discussed a determination of the biological value of a basal ration similar to but not identical with the ration used in this work was made and is reported by Gerritz ('31). The basal ration used contained no animal protein supplement, and the per cent of protein was 11.4. The growth on this ration was below normal, the chicks placed on it at the age of 2 weeks having attained a weight of only 475 gm. at 11 weeks of age, in comparison with an average weight of 800 or 900 gm. at that age. However, the chicks seemed in good condition. The growth curve of a second lot, transferred from a commercial ration at 11 weeks of age, did not show the normal slope up to 15 weeks of age. The biological value of the basal ration each week between the age of 3 and 11 weeks was as follows: 65.75, 77.33, 78.47, 79.71, 80.15, 75.68, 70.63, 76.19, 56.05. The second lot started at 11 weeks of age, showed weekly biological values of 70.80, 75.85, 77.03, 81.98. With the younger group there was an increase up to 7 weeks of age, followed by a decline. The older group showed increasing values for the 5-week period. It is evident that the biological value of the basal ration used is materially

below that of the ration carrying the herring meal supplement when used in quantities sufficient to make the protein level 12 and 15 per cent. However, it is above the value obtained when the protein level was 23 per cent.

Bringing together the different factors discussed, it is apparent that the two high protein rations fed to lots 57 and 58 produce slightly better growth than that fed lot 56, and all three are markedly better than the low protein ration. The chicks on the latter ration consume less feed than the other three lots, which are about equal. The gain per gram of feed consumed is about equal for the three high protein lots, but less for the low protein lot. The gain per gram of protein consumed is greater for lot 56, the ration containing 15 per cent protein, although it is not strikingly above lots 55 and 57. Lot 58 shows decidedly less gain per gram of protein consumed. The biological value of the rations for lots 55 and 56 is approximately the same and distinctly above that for the two high protein lots. The biological value of the two low protein rations is also above that of the basal ration, while the high protein ration is below the basal ration. These facts support the conclusion that a protein level of about 15 per cent shows optimum efficiency for this ration when fed to growing chicks. Whether or not the somewhat increased growth obtained with higher levels of protein is desirable is still open to question. The study of this question is being continued. The value of fish meal for older chicks is being studied and will be reported later. The digestibility of protein by chicks was not influenced to any marked extent by the level of protein intake as was shown by Swift, Black, Voris, and Funk ('31) using a protein supplement composed of 75 per cent dried buttermilk, 12.5 per cent fish meal, and 12.5 per cent meat scrap.

The efficiency of the fish meal in promoting growth seems to be high, as shown by the gain per gram of protein and the gain in bird weight. The gain per gram of protein consumed is approximately equal to that for dry skim milk at the different levels, as reported by St. John, Carver, et al. ('30),

and Carver, St. John, et al. ('32) and is above that for meat scrap as reported by Norris and Heuser ('30). Growth was also above that reported by Swift, Black, Voris and Funk ('31) using a mixed supplement of buttermilk, fish meal and meat scrap. The weight of the chicks at 12 weeks was above that of the chicks fed dry milk and was from 200 to 300 gm. above that of the chicks fed meat scrap.

SUMMARY

The results of a study of the supplementary value of herring fish meal fed at different levels in an all-mash ration for growing chicks are given. The biological value of these rations is high. It decreases somewhat as the level of total protein increases. The chicks receiving the ration containing 15 per cent total protein showed a slightly more efficient use of protein based on the gain per gram of protein consumed. Increased percentage of protein was accompanied by increased rate of growth.

Based on growth, feed utilization, protein utilization, and biological value, the 15 per cent protein level in this all-mash ration appears to be more efficient under the conditions used than the other protein levels used.

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THE VITAMIN B AND VITAMIN G CONTENT OF BOSC PEARS. USE OF THE MUNSELL METHOD OF ASSAYING FOODS FOR VITAMIN G

RUTH DOUGLASS, MAE HALLOWAY, JESSAMINE C. WILLIAMS, AND ALTA GARRISON

Foods and Nutrition, School of Home Economics, Oregon State College

TWO FIGURES

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The Bosc pear is a variety particularly adapted to growth in Oregon and is produced abundantly in the southern part of the state. Its production is being studied at the Oregon Agricultural Experiment Station and extensive work is being done to establish markets in the East for the fruit. A mineral analysis of the Bosc pear has been completed in the department of chemistry and a Purnell research project has been conducted in the department of foods and nutrition to determine the most satisfactory methods of baking and serving Bosc pear (Kolshorn, '31). A study of the vitamin content seemed worthwhile, since no such studies have been made on this variety of pear.

The vitamin A and vitamin B content of Bartlet pears, raw and canned, reported by Kramer, Eddy and Kohman ('29) indicated that 5 to 7 gm. per rat per day was sufficient to maintain life without significant growth over a 60-day period, but there was a vitamin deficiency manifest in all the animals receiving these amounts. Neither in this investigation nor any other subsequent one have the components of the vitamin B complex been determined in pears. The studies here reported deal not only with the vitamin B complex, but with the content of vitamins B and G of Bosc pears.

EXPERIMENTAL

Preparation of the pears

The Bosc pears used in these experiments were harvested, ripened and stored under carefully controlled conditions, in accordance with the procedure now used for market practices. The fruit was grown at the College Experiment Station at Medford, Oregon, harvested and stored at a temperature of 32°F. The pears were ripened in 6 days at a temperature of 60° to 68°F. in a special ripening chamber, then held for 2 days in storage at a temperature of 32°F. when they were placed in a tunnel recirculation prune drier with a dry air circulation draft of about 600 feet per minute. Drying was accomplished in approximately 64 hours at a temperature averaging 160°F. (never in excess of 165°F.). After drying they were tightly sealed in no. 2½ cans and opened only for immediate use. The moisture content taken from sufficient samples of the dried product was found to be 9.67 per cent. The average moisture content of the fresh fruit was 80.17 per cent. Accumulative evidence that vitamin B is unaffected by drying led to this choice of procedure in preparing the pear for feeding tests (Sherman and Grose, '23).

Method. Healthy, normally growing albino and pied rats were weaned at 21 days and placed upon the experimental diets at 28 days when the average weight was about 40 gm. With the exception of two litters on Steenbock's ('23) stock ration, the diets used in the colony were either Sherman's ('22) diet 13 or Smith and Bing's ('28) modification of this diet.

The animals were distributed on the various experimental diets with weight, sex, and ancestry considered, in order that any influence due to these factors might be equalized in each experimental group. From five to twelve animals were used in each series.

The basal ration was a modification of the Sherman and Spohn ('23) diet 107 and had the following composition: purified casein, 18 per cent; dextrinized cornstarch, 66 per

cent; butter fat, 8 per cent; Osborne and Mendel ('19) salt mixture, 4 per cent; cod liver oil, 2 per cent; and agar, 2 per cent. Similar diets have been shown to be adequate in every respect, except for vitamin B complex.

The casein was purified by the leaching method (Steenbock et al., '23) and a preliminary test showed this product to be comparable in purity to the B- and G-free casein obtained from the Harris Laboratories.[1] The cornstarch was dextrinized by a method described by Palmer and Kennedy ('27). The butter fat and the cod liver oil were incorporated in the basal ration which was stored in a refrigerator for daily use.

TABLE 1

Effects of feeding Bosc pear as a source of undifferentiated vitamin B

DIETS	NUMBER OF RATS	AVERAGE INITIAL WEIGHT (GRAMS)	LENGTH OF DEPLETION PERIOD (DAYS)	AVERAGE WEIGHT AT END OF DEPLETION (GRAMS)	AVERAGE GAIN OR LOSS PER WEEK (GRAMS)	AVERAGE NET GAIN OR LOSS PER GRAM OF FOOD PER WEEK (GRAMS)	PER CENT POLYNEURITIC
Basal diet alone (vitamin B complex-free)	7	48	...	...	— 2.0	— 0.15	..
Basal diet + 0.5 gm. dried whole yeast	6	38	14.5	43.5	+ 13.3	+ 0.24	..
Basal diet + 1.0 gm. dried Bosc pear	8	41	14.0	44.5	+ 1.3	+ 0.02	87
Basal diet + 1.5 gm. dried Bosc pear	8	41	14.0	44.5	+ 4.2	+ 0.14	..
Basal diet + 1.75 gm. dried Bosc pear	5	43.2	14.2	47.4	+ 4.8	+ 0.20	..

Vitamin B complex in Bosc pears

All animals were 'depleted' of body storage of vitamin B complex by placing them on the basal ration until a stationary weight or a slight loss of weight was noted. The average time of this depletion period was 14 days (table 1).

Preliminary tests indicated the use of at least three levels of dried pear for vitamin B complex determinations, namely,

[1] The Harris Laboratories Inc., Tuckahoe, New York.

1.0 gm., 1.5 gm. and 1.75 gm., equivalent to 8.29 gm., 12.44 gm. and 14.57 gm. of fresh pear, respectively. Normal growth was obtained when the basal ration was supplemented by 0.5 gm. of dried yeast[2] daily (table 1), thus serving as a positive control while the basal ration alone produced the usual decline and death and thus served as a negative control.

Results. When 1.0 gm. of dried Bosc pear was fed daily as a source of the vitamin B complex, polyneuritic symptoms were noted with little exception and the average gain per week through the 8 weeks' experimental period was only 1.31 gm. while the gain per week on the basal diet supplemented by 0.5 gm. of dried yeast furnishing the vitamin B complex was 13.3 gm. A progressively better growth resulted on the higher levels of dried pear (table 1). No tests were made to discover what amount of dried pear would furnish sufficient quantities of the vitamin B complex to produce normal growth.

Expressing the results in Sherman units ('32), this limited number of tests indicates that 1.21 gm. of dried pear (10.03 gm. of fresh pear) furnish one unit of vitamin B complex.

Vitamin B (B_1) in Bosc pears

To determine the vitamin B (B_1) in Bosc pears the same basal ration was used supplemented by autoclaved yeast as a source of vitamin G (B_2). Methods and results vary considerably regarding the time and temperature used in autoclaving to destroy vitamin B (B_1) and retain the maximum quantity of vitamin G. It is now well known that the hydrogen ion concentration influences the retention of the vitamin G factor. Chick and Roscoe ('30) found that yeast at the normal hydrogen ion concentration autoclaved for 5 hours at 120°C. contained only 50 per cent of the vitamin G present originally and was apparently free of vitamin B. The yeast used in the present investigation was autoclaved for 5 hours at 120°C. and when fed at pH 5 at a level of 0.5 gm. daily it furnished enough vitamin G for normal growth when vitamin B (B_1) was supplied.

[2] Obtained from Standard Brands Co., New York City.

Supplements of dried pear were fed at three different levels 1.0 gm., 1.5 gm., 1.75 gm., respectively, as a source of vitamin B (B_1).

Results. The results are shown in table 2 and chart 1. That the autoclaving of the yeast was effective in destroying vitamin B (B_1) was demonstrated, since animals on the basal diet plus 0.5 gm. autoclaved yeast declined and death occurred

TABLE 2

Effects of feeding Bosc pear as a source of vitamin B (B_1)

DIETS	NUMBER OF RATS	AVERAGE INITIAL WEIGHT (GRAMS)	LENGTH OF DEPLETION PERIOD (DAYS)	AVERAGE WEIGHT AT END OF DEPLETION (GRAMS)	AVERAGE GAIN OR LOSS PER WEEK (GRAMS)	AVERAGE NET GAIN OR LOSS PER GRAM OF FOOD PER WEEK (GRAMS)	PER CENT POLYNEURITIC
Basal diet + 0.5 gm. autoclaved yeast (vitamin B (B_1) free	10	38	14.2	42.5	— 1.6	— 0.05	20
Basal diet + 0.5 gm. autoclaved yeast + 1.0 gm. dried Bosc pear	10	37.3	14.5	44.8	+ 3.0	+ 0.12	80
Basal diet + 0.5 gm. autoclaved yeast + 1.5 gm. dried Bosc pear	12	42.0	15.2	46.6	+ 5.5	+ 0.21	..
Basal diet + 0.5 gm. autoclaved yeast + 1.75 gm. dried Bosc pear	5	44.2	14.4	44.8	+ 8.3	+ 0.24	..

at the same time as animals fed the basal diet alone. When 1.0 gm. of dried Bosc pear plus 0.5 gm. autoclaved yeast were fed as supplements to the basal diet, an average gain of 3.0 gm. per week was obtained. While this amount of pear produced a small gain in weight, it did not prevent the occurrence of polyneuritic symptoms in any except two animals in this group. The animals showed weakness and head retraction and severe convulsions occurred in four animals. Autopsy

findings gave evidence of a vitamin B deficiency. That 80 per cent of the animals developed polyneuritis on the 1.0 gm. level of pear, while only 20 per cent demonstrated these

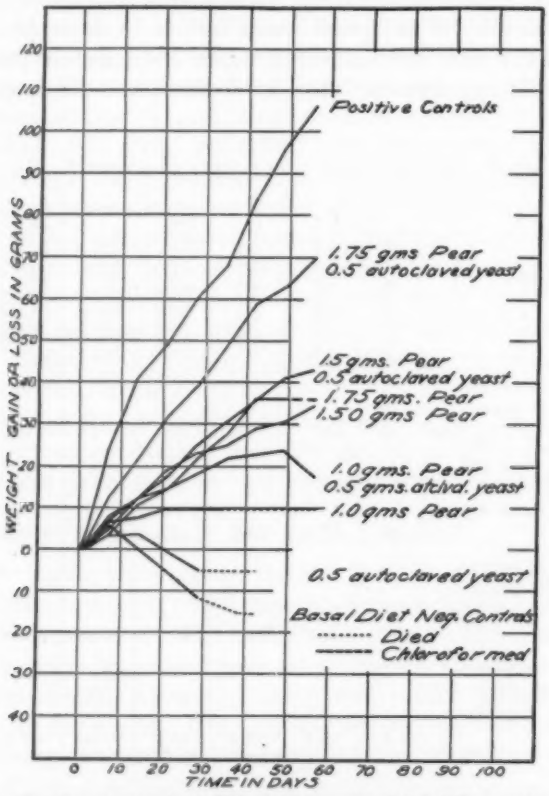

Chart 1 Results on growth of different amounts of dried Bosc pear as sources of vitamin B complex and vitamin B.

symptoms when no pear was fed is a fact concordant with the observations in similar investigations. Death often occurs too quickly on a diet entirely devoid of vitamin B for the animal to show symptoms characteristic of this deficiency.

With 1.5 gm. of dried pear as a supplement and 0.5 gm. of autoclaved yeast, there was an average weekly gain of 5.5 gm. and no symptoms of polyneuritis were manifested. Autopsy showed normal organs and no curvature of the spine.

The average weekly gain on the 1.75 gm. supplement of dried pear was 8.3 gm. Some difficulty was experienced at first in inducing the animals to consume this daily portion of pear. However, by removing the basal diet until the pear was consumed, the difficulty was eliminated. The animals in this group compared favorably with the positive controls (dried yeast supplement) in appearance, but failed to make more than 62 per cent of the gain made by the controls.

A progressively better growth resulted when levels of 1.0, 1.5, and 1.75 gm. pear were fed as a source of vitamin B (B_1), although none of these levels approximated normal growth. The food intake of the animals on the three levels of pear decreased per gram of rat as the supplements of pear increased, indicating that where the larger quantity of pear was consumed it replaced some of the basal ration (table 2).

Using the Sherman unit for expressing the vitamin B (B_1) content, the above results indicate that 0.73 gm. of dried Bosc pear (6.0 gm. of fresh pear) contains one unit of vitamin B. This estimate is based on gains in growth only and does not measure the polyneuritic or other symptoms of a deficiency. In view of our present limited knowledge of the various factors of the undifferentiated vitamin B, this quantity of pear may carry other unknown factors than vitamin B (B_1). Recently, Sherman ('31) has stated that—

> in view of recent evidence that after several weeks on the basal diet, other vitamins, not yet well defined, may begin to be the limiting factors also, and thus complicate the measurement of Vitamin B, it now seems perhaps better to base the quantitative comparison upon the rate of gain during a period of four weeks beginning at the end of the depletion period.

Vitamin G (B_2) in Bosc pears

Progress in the study of vitamin G values in foods has been hampered by the fact it has been necessary to prepare extracts of a food rich in vitamin B and low in vitamin G, using a solvent which removes only a minimum amount of G. This involves much time and expense and it was practically prohibitive in this laboratory, nor was it possible to purchase vitamin B extracts free of vitamin G.

Through private communication with Dr. Hazel Munsell, of the Bureau of Home Economics, U. S. Department of Agriculture, information was obtained regarding the use of white corn in a diet as a source of vitamin B (B_1) but furnishing very little vitamin G. Later, Munsell published her results ('31), showing "that 30% of white corn in the Sherman and Spohn Vitamin B free diet does not supply an amount of vitamin G sufficient to promote growth or prevent the occurrence of symptoms of pellagra."

White corn was therefore used to the extent of 30 per cent of our basal diet which was identical with the Sherman and Spohn diet, with the exception of 2 per cent agar replacing 2 per cent of the starch. In comparing white corn with wheat as sources of B and G, Munsell found it to be slightly poorer than whole wheat in both vitamin B (B_1) and vitamin G. On a diet in which the vitamin G carried by 30 per cent white corn was the limiting factor, the growth of the young rats was shown to be proportional to the vitamin G of the test food, fed in addition to the basal diet. It is understood that in the assaying of foods for vitamin G values, all growth rates in the experimental period must be compared to that obtained on the basal diet containing a minimum quantity of vitamin G in the 30 per cent white corn.

Method. The variety and source of the white corn was the same throughout the experiment.[3] Other constituents of the diet were prepared as for the assays of vitamin B complex and B. The experimental animals were from the same stock

[3] Champ, White Pearl, Portland Seed Company, Portland, Oregon.

with the same breeding and nutritional history. The average weight of the animals at 28 days when put upon the experimental diets was 39 gm.

The depletion period ranged from 7 to 16 days. Five to eleven animals were used in each group. A few experimental animals were continued 50 per cent longer than the experimental period, to ascertain the effects of a longer period of vitamin G deficiency.

The animals were divided into nine groups as follows:

Group I—Basal diet A (30 per cent corn).
Group II—Basal diet B (B and G free).
Group III—Basal diet A plus 0.5 gm. autoclaved yeast.
Group IV—Basal diet A plus 0.5 gm. autoclaved yeast and 0.5 gm. dried pear.
Group V—Basal diet B plus 0.5 gm. of dried yeast.
Group VI—Diet A plus 0.5 gm. dried pear.
Group VII—Diet A plus 0.75 gm. dried pear.
Group VIII—Diet A plus 1.0 gm. dried pear.
Group IX—Diet A plus 1.5 gm. dried pear.

Groups I and II constitute negative controls and were established because it was desired to secure symptoms of G avitaminosis, but primarily to test the vitamin G deficiency in the basal diet. Groups III, IV and V were planned as three positive controls and to determine whether basal diet A was adequate when vitamin G was supplied. Groups VI, VII, VIII, and IX were planned to test the various levels of pear fed as supplements to basal diet A as a source of vitamin G.

Results. The fact the average weekly gain was 1.92 gm. for group I (table 3) indicates that the constituents of the diet are not entirely free from vitamin G or that some other growth factor is present, or that the experimental animals were not depleted of the bodily reserves of a growth promoting vitamin. There are two possible sources for vitamin G in basal diet A, the casein and the white corn. Since the casein used was the same as that used in the purified diet (basal diet B) which produced a weekly loss in weight of 1.8 gm. (table 3, chart 2), death resulting, the presence of a very small amount of vitamin G must be assigned to the white corn as Munsell has reported ('31). The rats were considered depleted of their

store of vitamin G when the weight became stationary or there was a slight loss of weight. In group I growth was restricted to such a low level that when autoclaved yeast, a proved source of vitamin G, was added, the rate of growth increased 87 per cent.

TABLE 3

Effects of feeding Bosc pear as a source of vitamin G (B_2)

GROUPS	NUMBER OF RATS	AVERAGE INITIAL WEIGHT (GRAMS)	LENGTH OF DEPLETION PERIOD (DAYS)	AVERAGE WEIGHT AT END OF DEPLETION (GRAMS)	AVERAGE GAIN OR LOSS PER WEEK (GRAMS)	AVERAGE NET GAIN OR LOSS PER GRAM OF FOOD PER WEEK (GRAMS)
I. Basal diet A (vitamin B (B_1) supplied in 30 per cent white corn)	7	41.0	12.0	49.4	+ 1.9	+ 0.06
II. Basal diet B (vitamin B complex-free)	5	47.0	...	...	— 1.8	— 0.07
III. Basal diet A + 0.5 gm. autoclaved yeast	11	42.0	12.0	49.0	+ 16.0	+ 0.24
IV. Basal diet A + 0.5 gm. autoclaved yeast + 0.5 gm. dried pear	8	41.6	14.0	53.4	+ 19.0	+ 0.30
V. Basal diet B + 0.5 gm. dried yeast	7	36.0	15.0	48.5	+ 15.1	+ 0.22
VI. Basal diet A + 0.5 gm. dried pear	8	39.9	11.0	48.9	+ 5.2	+ 0.25
VII. Basal diet A + 0.75 gm. of dried pear	7	35.4	11.0	45.3	+ 7.5	+ 0.17
VIII. Basal diet A + 1.0 gm. dried pear	6	41.0	11.0	49.7	+ 6.3	+ 0.17
IX. Effect of basal diet A + 1.5 gm. dried pear	6	38.0	9.0	44.0	+ 9.0	+ 0.20

Severe symptoms of G avitaminosis reported by numerous investigators were not obtained, although some characteristic symptoms were noted. During the 8 weeks' experimental period, the most easily discernible reactions, aside from re-

tardation in growth were sluggishness of movements and a lowering of vitality. Among the rats kept on the vitamin G deficient diet over a period of several months, the skin became dry and the fur lost its luster and fell out easily. Ears,

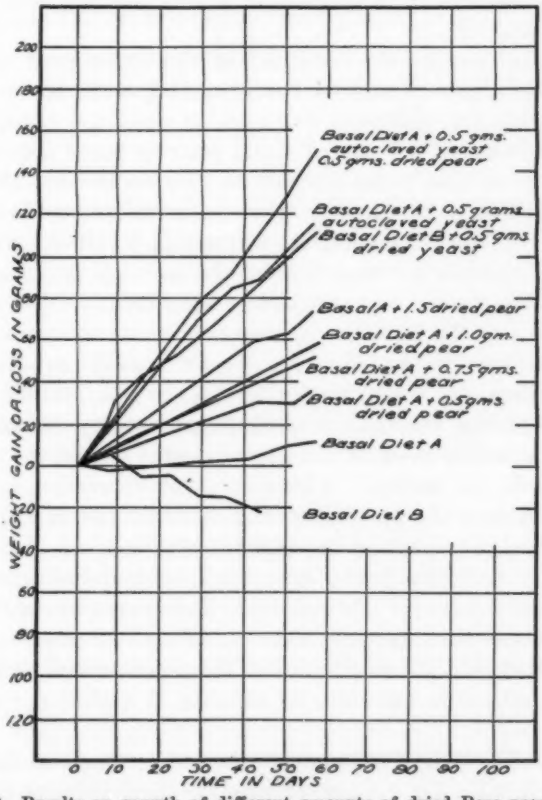
WEIGHT GAIN OR LOSS IN GRAMS
TIME IN DAYS
Basal Diet A + 0.5 gms. autoclaved yeast 0.5 gms. dried pear
Basal Diet A + 0.5 grams autoclaved yeast
Basal Diet B + 0.5 gms. dried yeast
Basal A + 1.5 dried pear
Basal Diet A + 1.0 gm. dried pear
Basal Diet A + 0.75 gms. dried pear
Basal Diet A + 0.5 gms. dried pear
Basal Diet A
Basal Diet B

Chart 2 Results on growth of different amounts of dried Bosc pear as sources of vitamin G.

tail and feet assumed a bluish color. It has been shown that if the diet contains a little vitamin G, death is postponed and a dermatitis develops slowly (Sherman and Sandels, '31). Since our basal diet contained a minimal amount of vitamin G

in the white corn, this may account for the slow development of 'these' symptoms.

Group II furnishes evidence that the casein purification was satisfactory and that the animals' reserve store of vitamin G was soon exhausted if placed on a diet lacking this factor (table 3, chart 2).

Group III proved the efficiency of the autoclaved yeast to furnish sufficient vitamin G for normal growth when fed at a level of 0.5 gm. daily.

The addition of 0.5 gm. of dried pear to basal diet A plus 0.5 gm. autoclaved yeast (group IV) indicates that the pear was supplying some limiting growth factor when the weight gains for the 8-week period are compared, but if the comparison be confined to a 5-week period the average gain per week of these two groups was practically identical.

Sherman ('31) tentatively suggests that all quantitative determinations be based on a 4- or 5-week experimental period, since the rate in gain or loss is maintained fairly uniform during the first 4 weeks, and usually between the fifth and seventh week of the experimental period the growth curve tends to flatten. Therefore, interpretation of data should consider the possibility of a limiting factor appearing during the latter part of the experimental period of 8 weeks.

Group V was introduced as a check against the other positive controls, groups III and IV. The respective gains per week in these three groups were 15, 16 and 19 gm. (table 3). It is evident that the purified diet B supplemented with yeast supplied adequate amounts of vitamin B and G for normal growth.

Groups VI, VII, VIII, and IX show the effects of the addition of 0.5, 0.75, 1.0, 1.5 gm. of pear, respectively, as a source of G to basal diet A (table 3, chart 2). The gains in weight of animals in these groups were in proportion to the amounts of pear fed. It may be assumed, therefore, that the dried pear was supplying vitamin G. Translating these data into Sherman-Bourquin units, it appears that 0.59 gm. of dried pear (4.64 gm. of fresh pear) contain a unit of vitamin G

and also comparing Bosc pear as a source of vitamin G with autoclaved yeast, it would require 2.97 gm. of dried pear (24.87 gm. of fresh pear) fed daily, to produce the same rate of growth as 0.5 gm. of autoclaved yeast. In other words, autoclaved yeast is approximately sixty times as good a source of vitamin G as dried Bosc pear.

CONCLUSIONS AND SUMMARY

1. The vitamin B complex, vitamin B and vitamin G, of Bosc pears has been studied and tentative units for each are suggested.

2. Expressed as the quantity of dried pear yielding one Sherman unit, 1.21 gm. (10.03 gm. fresh pear) will yield 1 unit of vitamin B complex. Translating this in terms of units per ounce, 1 ounce of fresh pear furnishes 2.8 units of vitamin B complex.

3. As a source of vitamin B, 0.73 gm. dried Bosc pear (6.0 gm. fresh pear) furnishes 1 unit of vitamin B, or 1 ounce fresh pear furnishes 4.7 units of vitamin B.

4. As a source of vitamin G, 0.59 gm. of dried pears (4.64 gm. of fresh pear) contains 1 unit of vitamin G, or 1 ounce of fresh pear furnishes 6.1 units of vitamin G.

5. It appears, therefore, that Bosc pears are a somewhat richer source of vitamin G than vitamin B.

6. Results show that white corn, when fed as 30 per cent of basal diet B, supplied enough vitamin B to prevent symptoms of polyneuritis and vitamin B deficiency, but does not supply enough vitamin G to promote more than very slight growth (1.9 gm. weekly).

7. Autoclaved yeast at pH 5 when fed at a 0.5 gm. level, supplemented the 30 per cent white corn in vitamin G and normal growth was obtained on this level.

8. When vitamin B is adequately supplied by white corn, vitamin G is furnished by Bosc pear in the same ratio as the quantity of pear supplied in the diet.

9. In laboratories not equipped to prepare extracts of vitamin B for use in the assaying of foods for vitamin G, fairly satisfactory results may be obtained by use of the Munsell method in which white corn furnishes the vitamin B.

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FAT SOLUBLE VITAMINS

XXXV. THE OPHTHALMOGENIC PROPERTIES OF CERTAIN RATIONS LOW IN VITAMIN A [1]

CARL BAUMANN AND HARRY STEENBOCK
Laboratory of Agricultural Chemistry, University of Wisconsin, Madison

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Ophthalmia, as a characteristic symptom of vitamin A deficiency, is subject to variations in its occurrence, as is evident from the reports of workers in different laboratories. Osborne and Mendel ('21) report an incidence of ophthalmia in 50 per cent of their animals; Stephenson and Clark ('20), 65 per cent; Emmett ('20), 98 per cent, and Sherman and Munsell ('25), 95 per cent. In our experience during the last 3 years, during which we fed approximately 500 rats on a ration uniformly free from vitamin A, we observed an incidence of ophthalmia in 70 per cent of all animals at the end of 5 weeks. This figure was increased to 95 per cent at the end of 6 weeks. As a matter of fact, many papers dealing with biological assays for vitamin A make no mention of the incidence of ophthalmia, presumably because such symptoms were either very mild or entirely absent. Of course, some of the variations in the experience of different laboratories may have been due to difference in the storage reserves of the animals, which may have been sufficient to delay the onset of all symptoms of deficiency beyond the period of observation; but this does not explain why symptoms of ophthalmia should have failed to appear before death from vitamin A deficiency ultimately resulted. It is possible that

[1] Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

the strains of rats used in some laboratories may have had a predisposition to ophthalmia, and that other strains, in turn, may have possessed a primary weakness in certain organs leading to an early failure of function and thereby death. We, for instance, were led to suspect such a situation when at one time in a number of litters the majority of the animals died precipitously from hemorrhages in the pancreas without any other indications of lack of vitamin A. This experience, however, has not been duplicated within the last year or more.

The high incidence of ophthalmia in our work in contrast with the observations made in other laboratories led us to study the specific role of various dietary factors which might be responsible. In these studies we varied the composition of our basal starch ration by substituting for the starch various grains and their by-products and by putting in yeast, agar, various salts, and additional protein. The protein was added not only to determine whether it had any direct effect, but also to determine the effect of better growth which it made possible. The yeast was put in for the same reason, and the salts were modified to determine if by chance the action destructive of vitamin A, well known to be exerted by iron salts, for instance, would reveal the presence of vitamin A in the basal diet. In some of these rations we incorporated the cereal ingredients after they had been cooked at a high temperature, such as is used in the preparation of our cooked starch, which has been a common ingredient in our usual basic vitamin A-free diet. In other cases we moistened the ration with water or mixed it with oil to make it less dusty, and thereby possibly less irritating in an incipient ophthalmic condition.

We shall not present all of our results, inasmuch as many of them have no bearing on the ultimate outcome. But, in general, we can state that as a result of these experiments we have come to the conclusion that other factors besides the mere freedom of the ration from vitamin A may be responsible not only for an acceleration in the rate at which ophthalmia

develops, but also for the severity which it attains in the course of the first few weeks. Although our experiments were not designed to reveal whether or not these factors would have determined the absolute occurrence or non-occurrence of ophthalmia if the experiment had been sufficiently prolonged, yet we are of the opinion that in some cases death ultimately might have resulted entirely from causes other than ophthalmia.

EXPERIMENTAL

Our experiments were carried out with young rats weighing 40 to 50 gm. at an age of 22 to 27 days. They were so distributed among the different lots of each series that litter representatives were present in each lot. Each lot was composed of five animals. The animals were weighed weekly until deficiency symptoms appeared—after that they were weighed at more frequent intervals. The rations were fed in tin feeding cups about 2½ inches high and 3½ inches in diameter. Vitamin D was furnished in all rations by the addition of 0.1 per cent of yeast which had been irradiated so as to contain one rat unit per milligram (Steenbock, et al., '32). In some cases records of the food consumed by each lot were kept for short periods. The animals were carefully examined for ophthalmia every few days after the first 3 weeks. For purposes of comparison the ophthalmias were arbitrarily classified as given in the footnote to table 2.

After the symptoms of vitamin A deficiency had become pronounced, carotene was administered in some cases for the purpose of determining the curative and growth promoting properties of the ration when vitamin A activity was present. We entertained the hope that these data would enable us to select a ration in which certain variables would have been omitted and the ration thereby made more suitable for vitamin A assays. Our experiments will be discussed in series which were run consecutively as the problem developed.

Series 1. Comparison of different cereal grains. This series actually consisted of two series. They were run con-

secutively to verify the original observations, but the results are presented collectively to save space. In these experiments we made a comparison between rolled oats, white corn, wheat, polished rice, and cornstarch for their ophthalmogenic and growth promoting properties. Ration F (table 1), containing the cornstarch, represented our most commonly employed ration which we have frequently designated as our basal synthetic ration for vitamin A work. In this the starch had been cooked by autoclaving with water at a temperature of 117° for 2 hours. It had then been dried thoroughly at 60° for 1 week. The casein was a commercial product in which

TABLE 1

Composition of rations[1]

		CASEIN	NaCl	$CaCO_3$	SALTS 40	YEAST	AGAR
(a) Rolled oats	86	12	1	1	..	..	..
(b) Rolled oats	80	18	1	1	..	..	..
(c) White corn	86	12	1	1	..	..	..
(d) Wheat	86	12	1	1	..	..	..
(e) Polished rice	80	12	1	1	..	6	..
(f) Cornstarch (cooked)	69	18	..	..	4	7	2

[1] All rations contained 0.1 per cent of dried yeast obtained from standard brands and irradiated in our laboratory so as to contain one unit (Steenbock) of vitamin D per milligram.

all vitamin A had been destroyed by heating it in a finely ground condition in thin layers in shallow pans for 2 weeks at 90°C. The composition of the salt mixture has already been described (Steenbock and Nelson, '23). The yeast[2] had been previously tested for vitamin B potency in our laboratory.

In table 2 there are presented the results on the production of ophthalmia and the changes in weight of all animals after they had been on experiment from 26 to 35 days. As seen from the table, in series 1 there occurred pronounced growth of animals on the rice ration, together with an early incidence of ophthalmia. In this respect the cornstarch ration was a

[2] Obtained from Northwestern Yeast Co., Chicago

TABLE 2

Variations of ophthalmia and growth on different rations

LOT NOS.	RATIONS	OPHTHALMIC TOTALS [1]						WEIGHTS (GRAMS)				
Series 1. Comparison of grains												
		(28)	(33)	(35)	(38)	(40)		(28)	(33)	(35)	(38)	(40) [2]
161, 167	Rolled oats (12 per cent casein)	0	0	9	11	11		84	85	84	84	84
162, 168	Rolled oats (18 per cent casein)	0	0	5	8	8		82	84	83	83	84
163, 168	White corn	0	0	2	3	3		86	87	86	85	85
164, 170	Wheat	0	0	0	0	1		108	112	113	112	113
165, 171	Polished rice	7	14	15	..	..		121	118	110	...	...
166, 172	Cornstarch	0	7	11	..	..		106	109	107	...	...
Series 2. Effect of 6 per cent yeast												
		(33)	(35)	(37)	(38)	(40)	(42)	(33)	(35)	(37)	(38)	(42)
190	Rolled oats (12 per cent casein)	4	4	6	6	7	8	97	98	99	100	102
191	Rolled oats (18 per cent casein)	0	2	6	6	7	7	124	128	128	133	131
192	White corn	2	5	8	8	..	..	122	119	128	116	...
193	Polished rice	12	12	15	15	..	..	103	100	97	96	...
194	Cornstarch	2	2	4	8	9	..	108	109	109	112	105
195	Cornstarch (9 per cent casein)	0	0	4	..	..	12	78	...	80	...	...
Series 3. Rice from different sources												
		(26)	(28)	(30)	(35)			(26)	(28)	(30)	(35)	
200	Cornstarch	0	0	4	13			108	108	107	108	
201	Polished rice no. 1	3	7	14	..			104	104	98	88	
202	Polished rice no. 2	4	5	10	13			124	126	123	107	
203	Polished rice no. 3	8	12	14	..			119	123	114	111	
204	Polished rice no. 4	3	4	8	..			124	118	102	...	
205	Polished rice no. 5	4	11	14	..			119	120	104	96	
Series 4 and 5. Effect of heat, water, or fat												
		(28)	(30)	(32)	(35)			(28)	(30)	(32)	(35)	
234	Rice starch cooked	0	0	0	2			83	85	86	89	
236	Cornstarch	0	1	2	6			118	119	122	122	
237	Polished rice	8	9	11	..			132	130	130	...	
238	Polished rice (fed moistened)	0	0	1	2			117	...	124	...	
241	Polished rice (cooked)	0	0	0	0			58	...	55	53	
242	Polished rice (plus oil)	0	0	4	8			129	...	132	125	
243	Cornstarch (plus oil)	0	0	2	7			127	...	131	133	

[1] Ophthalmic totals were obtained by multiplying the degree of ophthalmia by the number of animals afflicted in each lot. To make all the data comparable the values for series 1 were divided by 2, because there were ten animals in each group instead of five. The degrees of ophthalmia were differentiated arbitrarily as follows: First degree, bareness of lids with or without exudate; second degree, bareness of lids with slight swelling or slight bleeding; third degree, marked swelling of lids with or without hemorrhage and with or without necrosis of conjunctiva; fourth degree, purulency of the eyeball together with any or all of the aforementioned symptoms.

[2] The figures in parentheses represent the number of days that the animals had been on experiment.

close second. Wheat also gave good growth, but the incidence of ophthalmia was much delayed. This was in harmony with earlier results from this and other laboratories which have shown that the wheat kernel contains estimable quantities of vitamin A (Steenbock and Coward, '27; McCollum and Davis, '15). Rolled oats and white corn both allowed but little growth, and both produced ophthalmia somewhat slower than the cornstarch ration, although much earlier and more severely than the wheat. That the rice and cornstarch rations were suitably constituted to reveal differences in vitamin A content was revealed by the fact that both of them gave a favorable response to the addition of carotene. When 5 gamma carotene were given daily to each animal, the rice-fed animals gained 30 gm. in 4 weeks and the starch-fed animals 35 gm. With the corn ration the addition of carotene also gave good growth, but with the oat rations the results were mediocre. Growth on the ration containing 18 per cent casein was somewhat better than with 12 per cent casein.

Series 2. The effect of yeast additions. Inasmuch as in series 1 the combined results of earliest production of ophthalmia and the best growth had been obtained with the two rations, namely, rice and cornstarch, which also contained 6 to 7 per cent yeast, that series of experiments was repeated with the addition of yeast to the other rations as well. However, from this series the wheat ration was omitted as it obviously contained too much vitamin A to make its inclusion worth while. In these experiments the addition of yeast still left the polished rice ration outstanding for its early production of ophthalmia. Although growth was increased in the other rations, except where the protein level had been too low, it was made evident that the early appearance of ophthalmia was not directly related to the rapidity of growth.

Series 3. The effect of rice from different sources. To determine if the early production of ophthalmia is a property common to rice in general, we secured rice from five different

sources and fed it as usual in ration E (table 1). Ration F, containing cornstarch, was again used as a control. As shown in table 2, there occurred some variations in the incidence of ophthalmia and also in the production of growth; but we are rather doubtful as to their significance in view of the small number of animals employed. It can, however, be stated that all the rice rations were without question more ophthalmogenic than the control ration.

Series 4 and 5. The effect of various treatments of rice and various supplements. These series were run to determine if the physical state of the polished rice ration was responsible for its ophthalmogenic properties. To this end polished rice was fed in one ration in an untreated form, except for its being ground to an impalpable flour. For comparison therewith there were fed identical rations, except that in one case they contained cooked rice starch, in another case ground rice moistened with water, in a third case ground cooked rice, and in a fourth case ground rice mixed with 5 per cent cottonseed oil. For control purposes there was fed the usual cornstarch ration F with and without 5 per cent cottonseed oil.

A comparison of the degree of ophthalmia obtained from feeding the different rations showed rather striking results. As usual, ration E, containing the untreated ground rice, produced ophthalmia most quickly. When ration E was moistened and when it was treated with oil it produced ophthalmia at the same rate as the cornstarch ration. In other words, the addition of oil did not affect the incidence of ophthalmia on the starch ration, but it did retard it with the rice ration.

The growth results on these rations showed considerable variations. The best growth was obtained with the untreated rice ration or the untreated rice ration or cornstarch ration plus oil. The animals averaged 130 gm. in weight after 4 weeks. On the cornstarch ration alone, or on the raw rice ration fed moist, the animals averaged from 115 to 120 gm. in 4 weeks. On cooked rice practically no growth was obtained, the average weight after 4 weeks being 58 gm.

The inferences which might be drawn from the above findings are that the physical state of the rice was an important factor in determining its ability to produce ophthalmia. In such instances where the rice particles were made less irritating as a result of disintegration by cooking or by being weighted down with water or oil, ophthalmia was delayed in its appearance. It appeared very probable that the early incidence of ophthalmia on the rice ration was due to an irritating effect exerted by fine particles of starch which entered the eyes. As the evidence for this was entirely circumstantial, we decided to obtain further data on the exhaustion of vitamin A reserves by the vaginal smear test of Evans and Bishop ('22). This test is based on the fact that when the vitamin A stores of an animal become depleted cornified cells become persistent in the vagina. These can readily be followed in their occurrence by microscopic examinations of smears made daily.

Series 6. Vaginal smear experiments. Twenty-four rats weighing 50 to 60 gm. were divided into two groups. One group was given the cornstarch ration and the other the untreated polished rice ration. Vaginal smears were made as soon as the vaginal orifice made its appearance. This practice was continued for 2 weeks after cornified cells had persisted.

On the cornstarch ration of the twelve animals on experiment, nine showed their first smears characteristic of vitamin A deficiency in the period from the twenty-fifth to the thirty-eighth day. The average for the group was 31 days. Three animals remained immature. On the rice ration the first characteristic smears appeared from the twenty-fifth to thirty-sixth day, with an average of 30 days. One animal remained immature. These data show a remarkable uniformity in the appearance of the characteristic smears. However, on the rice ration ophthalmia again appeared earlier, and after 6 weeks the ophthalmic total, expressed according to the system used in table 2, was twenty-one for the rice ration and eight for the cornstarch ration. These results therefore indicate that while the vitamin A stores of the

animals on the two rations were depleted at the same rate, the rice ration without question possessed greater ophthalmogenic properties.

We also made the observation that our rice fed animals were more subject to diarrhea than the animals on cornstarch. On the cornstarch rations, diarrhea, as a matter of fact, was rarely observed and then only when the avitaminosis was far advanced. It might be proposed that the diarrhea was due to the coarseness of the rice particles and its high specific gravity, which made it more irritating. In reply to this it should be stated that while the specific gravity of the untreated rice ration was high, a rice flour ration was much lower; in fact, lower than the cornstarch ration, and yet both produced ophthalmia to an equal degree.

The relative coarseness of the cereal ingredients was not a factor, because the cereal products were all ground to a powder in a ball mill and microscopic examination failed to reveal any difference in shape of particles or sharpness of edges. Yet it was noteworthy that untreated rice was apparently digested with some difficulty because starch grains were frequently found in the feces.

SUMMARY

It appears as a result of our experiments that the physical qualities of a ration which may allow irritating ingredients to lodge in the eyes of an experimental animal are factors which should be given consideration in the production and possible cure of ophthalmia. As the incidence of ophthalmia frequently leads to a secondary effect on growth, the physical state of the ration may have an effect on growth as well. It is accordingly recommended that when rations low in vitamin A are to be used for assay purposes, they should be so treated by cooking, moistening, or otherwise, as to allay their dust producing properties. In addition it follows from our observations on growth and the production and cure of ophthalmia that a basal ration consisting of cooked polished rice 80, heated casein 12, sodium chloride 1, calcium carbonate 1, and

yeast, high in vitamin B, 6, constitutes an excellent basal ration for determining vitamin A in concentrates such as cod liver oil and the like. This ration, furthermore, has the advantage of low cost as its salt ingredients are especially inexpensive.

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THE EFFECT OF ACID ASH AND ALKALINE ASH FOODSTUFFS ON THE ACID-BASE EQUILIBRIUM OF MAN

FRITZ BISCHOFF, W. D. SANSUM, M. LOUISA LONG, AND MARGARET M. DEWAR

Chemical Laboratory, Potter Metabolic Clinic, Santa Barbara Cottage Hospital, Santa Barbara, California

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INTRODUCTION

While the evidence (Bischoff, '32) to date indicates that sustained changes in CO_2 content and plasma pH of humans outside the so-called normal limits cannot be produced by the ingestion of foodstuffs, the possibility and significance of changes within this limit have not been eliminated. Cullen and Earle ('29) have recently studied the normal changes in pH and CO_2 content of the blood during the course of the day. The normal shift in pH may be 0.07 more alkaline. The normal limits for venous blood plasma, according to these authors, is pH 7.33 to 7.51, and CO_2 content, 55 to 74 volumes per cent. Haldane ('21) showed that by taking 15 to 20 gm. of ammonium chloride per day an acidosis could be produced. He was able to lower the bicarbonate of his blood by one-half. This amount of ammonium chloride will reduce the pH of the blood by 0.2. The daily administration of 45 gm. of sodium bicarbonate was found necessary to change the reaction of the blood to a similar extent in the opposite direction. Smaller amounts of these substances upon administration will produce transitory effects (Palmer and Van Slyke, '17). It would require 18 pounds of oranges to have an alkaline ash content equivalent to 40 gm. of $NaHCO_3$, and 4.5 pounds of lean beef,

or 2 pounds of oysters, to have an acid ash content equivalent to 15 gm. of NH_4Cl.

Michalowsky ('30) has shown that the ingestion of 2.5 gm. of phosphoric acid daily, for a period of 6 weeks, had no effect upon the alkali reserve. The equivalent of lean beef in acid ash content is approximately a pound, so that Michalowsky's work does not rule out the possibility of producing a change in the blood by diet. Michalowsky also studied the effect of a mixed diet of meat, fat, and wheat on the alkali reserve. He observed no change. The exact composition of his diet is not available, nor the urine acidity. The foregoing facts raise the question as to the minimum amount of acid or alkali required to change the pH of the blood, it being pertinent to decide whether these amounts could be liberated in the consumption of a foodstuff.

The study of the effect of the ingestion of acid ash and alkaline ash foodstuffs on temporary shifts of the acid-base equilibrium of the body is complicated by the alkaline tide. In general, the acid and alkaline ash of the foodstuffs administered in this study have not been considered, an exception being the work of Fiske ('21) who found that the maximum alkalinity of the urine, after a protein meal, is often reached at a time when the excretion of phosphate and sulfate is at a maximum. Since fasting normal subjects show a diuresis with an alkaline tide in the morning (McCorvie, '25) and a fall in CO_2 combining power which may reach 5 volumes per cent, some doubt must be cast on the significance of experimental results which have apparently shown a relation between ingestion of foodstuff and change in acid-base equilibrium. In reviewing the older literature, one wonders whether the effects of exercise on the acid-base equilibrium were sufficiently controlled.

PLAN

The present study was concerned with two major problems: to determine whether it was possible to produce 1) a sustained shift or 2) a temporary shift in the acid-base equilibrium of

the blood following the ingestion of acid ash or alkaline ash foodstuffs.

The minimum amount of sodium citrate which would shift the acid-base equilibrium of the morning fasting blood significantly was determined and administered for 40 days. Sodium citrate was chosen because it is a natural alkaline ash-producing constituent of foods and introduces the factor of citric acid oxidation.

In another experiment, an individual was placed on his usual diet and then on a diet containing acid ash foodstuffs, and finally on a diet containing alkaline ash foodstuffs.

In a third experiment, the same individual was placed on an acid ash diet containing the acid ash equivalent of 3 pounds of beef. This diet was necessarily high in protein.

In studying temporary effects, that is, changes that might be induced during the course of 8 hours following the ingestion of a food, a pound of steak, a quart of milk, a pound of bananas or a quart of orange juice was eaten.

In all cases, except those in which the subject was required to maintain normal activity, blood samples were taken after a half hour of bed rest. In studying temporary changes, the subject was at bed rest during the entire course of the experiment, unless otherwise indicated. CO_2, oxygen content, and oxygen capacity were determined for whole blood. Plasma pH and CO_2 were also determined on plasma as drawn. For the corresponding intervals the urinary determination of titratable acidity, ammonia, and pH was made.

Since the primary interest was in plasma pH and alkali reserve, the data have been presented to show these values. The plasma bicarbonate is the best criterion of alkali reserve. In order that the comparison of all plasma bicarbonate values might be strictly comparable, the values presented were recalculated for completely oxygenated blood at the pH drawn, using the well-known constants and relations of Van Slyke ('31). In most instances, plasma CO_2 content was also determined, since this value serves as an excellent check for the whole blood data.

The analytical methods used were: whole blood CO_2, oxygen capacity and oxygen content according to Van Slyke and Neill ('24) and Van Slyke and Sendroy ('27), titratable acidity and ammonia according to Folin ('03) and Folin and Bell ('17), phosphorus according to Youngsberg ('30), sulfur according to Folin ('05). Plasma pH was determined by Cullen's method as modified by Bischoff, Long, and Hill ('30).

RESULTS

Normal variation. From four to seven pre-breakfast blood determinations were made at intervals for each of the six individuals studied. The greatest deviation between any two plasma bicarbonate values for any one individual was 3 m.Mol. The individual extreme values were: 26.9 to 29.2, 28.0 to 28.7, 28.5 to 30.0, 27.2 to 29.0, 24.0 to 27.0, 25.8 to 26.5. It would, therefore, be doubtful whether a 3-m.Mol. change in an isolated experiment, in which data were collected before and after the ingestion of a foodstuff, should be considered significant, as has been repeatedly done in the past. In the present studies, no change is considered significant if the values fall within the range established by a series of fasting values. Consecutive fasting values for a 3-hour interval were determined in two instances. Changes of a millimol were noted.

The normal variation of the individual's pre-breakfast pH covered 0 to 0.04 pH (three to to five determinations on each individual). The impression gathered in earlier studies of Bischoff, Long, and Hill ('30, '31) and Bischoff, et al., ('30) that each individual has a characteristic pH range is extended. It should be noted that, after an interval of 2 years, the values of subject L.C.M. shifted from the lower normal range to the upper range. The values for subject Mc. are the highest normal values so far found.

Effect of sodium citrate. The subject was hospitalized during the period of experimentation. The bleeding was done in the morning before breakfast. The first blood sample was collected for the determination of control values. The subject was then given 8 gm. of citrate per day for 7 days, when

blood data were collected. Following this period, citrate was given daily in 4-gm. doses, a blood sample being collected after 9 days. Thirty gm. of sodium citrate were then given daily for 40 days. Three blood samples were collected during this period, the first, 4 days after the initial dosing. Following this period, the basal diet was resumed for 10 days.

The low doses of citrate had no effect upon either the total CO_2 or blood pH. There was a remarkably close agreement for total plasma bicarbonate, the maximum variation being less than 1 m.Mol. per L. All the pH values but one were the same. The exceptional value was 0.05 pH more acid.

TABLE 1

Effect of sodium citrate administration upon the acid-base equilibrium of pre-breakfast blood

CITRATE ADMINISTRATION	PERIOD	URINE pH	BLOOD PLASMA [1]	
			$BHCO_3$	pH
			m.Mol. per L.	
Control	Initial	7.2	25.8	7.45
8 gm. citrate daily	7 days	7.8	25.6	7.40
Control	Initial	7.6	26.0	7.45
4 gm. citrate daily	9 days	8.0	25.8	7.45
30 gm. citrate daily	4 days	8.6	28.2	7.48
30 gm. citrate daily	22 days	8.4	27.9	7.48
30 gm. citrate daily	39 days	8.4	28.2	7.45
Control	10 days	6.8	26.5	7.45

[1] Recalculated for completely oxygenated blood at pH drawn.

These values were all within the normal established pH range. During the final period of sodium citrate administration, the plasma CO_2 rose 3 m.Mol. per L. The plasma pH rose 0.03 in two cases, with no change in a third. All these values are again within the normal range, but they indicate a shift to the alkaline side. One determination was made after breakfast and after the ingestion of 5 gm. of sodium citrate. The blood pH was elevated 0.03, the plasma bicarbonate 3 m.Mol. per L. over the basal values. These values are also in the normal range. The results are given in table 1.

Acid diet. The subject was asked to eat the diet he regularly followed for a period of 6 days. Twenty-four-hour

urine specimens were collected daily, the urine pH and titratable acidity being determined. Acid-base data were collected from blood drawn on two mornings before breakfast. The subject was then placed for 9 days on an alkaline ash diet. This diet consisted of a mixture of neutral and alkaline ash foods. Acid-base data of the blood were determined three times. An acid ash diet was then followed for 9 days. This diet consisted of a mixture of neutral and acid ash foods. Acid-base data for the blood were determined twice.

TABLE 2

Effect of ash variation in diet upon the pre-breakfast acid-base equilibrium of the blood

DIET	PERIOD	24 HOUR URINE		BLOOD FINDINGS (PLASMA)[1]	
		pH	Titratable acidity	pH	$BHCO_3$
			cc. N/10		*m.Mol. per L.*
Usual	6 days	5.8 ± 0.15	234 ± 27		
Usual	4th day	6.2	295	7.40	29.0
Usual	5th day	5.8	200	7.41	26.9
Basic	9 days	6.9 ± 0.15	130 ± 31		
Basic	2nd day	6.4	68	7.45	27.2
Basic	4th day	7.2	98	7.41	28.8
Basic	9th day	7.2	113	7.44	29.2
Acid	9 days	5.9 ± 0.12	262 ± 17		
Acid	4th day	5.8	273	7.40	28.5
Acid	9th day	6.4	246	7.44	29.2

[1] Complete oxygenation.

Probably the most interesting result of this experiment was that the urine pH during the uncontrolled diet period was as acid as that in the period when a carefully selected acid diet was consumed. The subject responded classically to the change in alkaline diet, the urine pH shifting from 5.8 to 6.9. No change in plasma pH or plasma CO_2 outside the normal expected variation was observed. The total CO_2 of the plasma was in the upper limits of normal, showing a maximum variation of 2.3 m.Mol. per L., the highest value being observed on the acid diet, the lowest on the control diet. The plasma pH fluctuated in the region of the lower limits of normal (table 2).

Since this experiment showed rather conclusively that fairly acid ash and alkaline ash diets had no effect upon the acid-base equilibrium of the blood, a more drastic experiment was planned. A period of 2 years elapsed between the first and second experiments. The same subject was studied. The diet consisted almost entirely of meat (exception 20 gm. of rice) with an acid ash equivalent of 3 pounds of lean beef. Brains, ham, chicken, veal, beef, and eggs were used. After 2⅔ days of this diet régime, the subject became nauseated, when he was returned to a more rational diet. It was regretted that a longer period on the high protein-acid ash diet could not be followed. If the response to ammonium chloride ingestion may be used as a criterion, an acid-base equilibrium would have been reached. The results showed no significant change in blood pH after 2 days on the diet. The 1 m.Mol. drop in plasma CO_2 might be significant, as it was the lowest value observed for the individual studied. Of great interest was the increase in total CO_2 the morning following the cessation of the acid diet. At this time a diarrhea developed. We have previously pointed out that Page ('23) observed an increase in alkali reserve in rabbits which developed a diarrhea. At this period the pH was still normal.

While the acid-base data of the blood again showed that the acid ash produced no decided shift, the other data which were gathered bear inspection. There was a lag in nitrogen excretion for a day and a half after the high protein diet was stopped (table 3). The blood urea nitrogen rose considerably. Ammonia excretion increased appreciably. No albumin or casts were found in the urine. These are all well-known observations.

Steak (temporary effect). Data for four individuals, including six experiments, were collected to determine the effect of the ingestion of 1 pound of steak (equivalent roughly to 50 cc. of normal acid). For subject Mc., a fall of 3 m.Mol. below the control value was noted at the eighth hour on two occasions, one under complete rest, the other under normal activity. However, 8 hours after the ingestion of a quart of

TABLE 3

Effect of ingestion of approximately 200 cc. normal acid as acid ash food

DATE	PERIOD	URINE FINDINGS					BLOOD FINDINGS			DIET				
		S.G.	pH	Alb. or casts	Total N-24-hr.	NH_3 24-hr.	Plasma $BHCO_3$[1]	Plasma pH	Urea N	Acid ash	Carbo-hydrate	protein	Fat	Calories
					Gm.	*Gm.*	*m.Mol./L.*		*Mg./100 cc.*	*Cc. N*				
May 11	Control	1.034	5.5	...	10.0		26.5	7.50						
May 12	Exp. diet	1.030	5.8	...	21.6	0.7				202	125	243	178	3080
May 13	Exp. diet	1.030	5.3	...	17.5	0.7				190	116	204	112	2293
May 14	Exp. diet	1.020	5.2	...	20.1	1.0	25.2	7.45	34.0	189	142	93	127	2090
May 15	Recovery	1.023	5.8	...	13.7	0.5	29.0	7.47	22.5					
May 16	Recovery	1.016	6.0	...	8.9	0.3								

[1] Recalculated for completely oxygenated blood at pH drawn.

milk (an essentially neutral ash food) a fall of 2 m.Mol. was noted (table 4). The maximum effect that could be attributed to the acid ash of the steak was therefore a decrease of 1 m.Mol. For subject B., a fall of 1 m.Mol. was noted the fifth hour, with a return to the control value the eighth hour. In this experiment subject B. was under normal activity. The experiment showed no change when subject B. was under rest. In the case of subjects H. and C., the decrease by 1 m.Mol. has no significance, since a similar fall was noted under fasting, following orange juice, or following 50 cc. of 7 per cent alcohol.

Orange juice and bananas. The four experiments concerned with orange juice or bananas showed no effect on the acid-base equilibrium. The small variation in values was well within the expected normal variation and was doubly controlled by taking fasting values at the same time of the day (table 4).

Urinary changes. Changes in the urinary constituents were determined during the periods of diet experimentation so that blood samples might be collected at the time when kidney excretion indicated maximum acid-base changes. In four out of the five experiments concerned with steak, the rate of phosphorus excretion was descending at the period of maximum urine acidity (table 5). It seems important to emphasize that there is no correlation between either urinary pH or titratable acidity and plasma bicarbonate. In the case of subject B. (five determinations), the plasma bicarbonate fluctuated between 27 and 28 m.Mol. at the time the urinary acidity was in the range 7.0 to 7.5; at the pH range 4.9 to 5.5, the bicarbonate fluctuated between 27 and 29 m.Mol (five determinations, table 4). Similar results were obtained for the other subjects studied and it seems unnecessary to present the correlation chart. In the case of subject B., the plasma bicarbonate values were the same when the rates of urinary excretion of titratable acidity and ammonia were markedly different (986 against 579 cc. 0.1 N acid per 24 hours). Rates of 106 and 230 showed identical plasma bicarbonate values

TABLE 4

Temporary acid-base plasma changes following ingestion of various foodstuffs

SUBJECT	PERIOD	CONDITIONS	PLASMA	
			$BHCO_3$[1]	pH
			m.Mol. per L.	
H.	8:30 a.m. Feb. 8	Fasting	28.0	7.46
	11:30 a.m. Feb. 8	Fasting	26.7	7.47
	8:50 a.m. Feb. 12	Fasting	28.5	7.48
	11:50 a.m. Feb. 12	3 hours after 1 quart of orange juice	26.5	7.49
	7:30 a.m. Feb. 18	Fasting	28.2	7.49
	1:30 p.m. Feb. 18	6 hours after 1 pound of steak	27.0	7.51
	9:00 a.m. Feb. 24	Fasting	28.7	7.47
	10:00 a.m. Feb. 24	1 hour after 50 cc. of 7 per cent alcohol	27.2	7.45
C.	9:00 a.m. Feb. 10	Fasting	26.1	7.49
	12:00 a.m. Feb. 10	Fasting	27.1	7.47
	9:00 a.m. Feb. 18	Fasting	24.0	7.49
	12:00 a.m. Feb. 18	3 hours after 1 quart of orange juice	26.0	7.50
	9:00 a.m. Feb. 25	Fasting	25.5	7.50
	4:30 p.m. Feb. 25	7.5 hours after 1 pound of steak	24.1	7.48
	9:00 a.m. Mar. 3	Fasting	26.0	7.47
	9:30 a.m. Mar. 3	0.5 hour after 50 cc. of 7 per cent alcohol	24.2	7.50
	9:00 a.m. Sept. 1	Fasting	26.3	7.51
	4:30 p.m. Sept. 1	7.5 hours after 1 pound of bananas	26.0	7.50
B.	9:30 a.m. Feb. 19	Fasting	28.0	
	10:30 a.m. Feb. 19	After 1 pound of steak	27.0	
	12:30 p.m. Feb. 19	After 1 pound of steak —at rest	28.8	
	2:30 p.m. Feb. 19	After 1 pound of steak	28.0	
	8:30 a.m. Aug. 26	Fasting	27.8	
	11:05 a.m. Aug. 26	After 1 pound of bananas	27.8	
	2:20 p.m. Aug. 26	After 1 pound of bananas —at rest	27.0	
	3:15 p.m. Aug. 26	After 1 pound of bananas	28.2	
	8:45 a.m. Aug. 31	Fasting	27.2	
	2:00 p.m. Aug. 31	After 1 pound of steak —normal activity	26.0	
	4:45 p.m. Aug. 31	After 1 pound of steak	27.2	
	8:30 a.m. Sept. 14	Fasting	29.0	
	2:30 p.m. Sept. 14	After 1 quart of milk—normal activity	28.0	
M.	7:00 a.m. Mar. 25	Fasting	30.0	7.50
	7:00 a.m. Mar. 26[2]	Fasting	29.2	7.53
	3:00 p.m. Mar. 26	After 1 pound of steak	26.2	7.49
	7:00 a.m. April 1	Fasting	29.5	7.52
	7:00 a.m. Aug. 19	Fasting	27.5	7.48
	11:00 a.m. Aug. 19	After 1 pound of steak	28.0	7.48
	3:00 p.m. Aug. 19	After 1 pound of steak	24.5	7.48
	7:00 a.m. Aug. 22	Fasting	29.5	7.49
	3:00 p.m. Aug. 22	After 1 quart of milk	27.5	7.49

[1] Recalculated for completely oxygenated blood at pH drawn.

[2] The morning following ingestion of 3 pounds of steak.

TABLE 5

Urinary findings for various diet periods

SUBJECT	PERIOD	CONDITIONS	URINARY FINDINGS AS HOURLY RATE					
			Titratable acidity	NH_3-N	P.	SO_3	N.	pH
			Cc. N/10	*Mg.*	*Mg.*	*Mg.*	*Gm.*	
Mc.	11:00 p.m. to 7:00 a.m. Mar. 25, 26	Fasting	28	40	59		1.17	5.1
	7:00 a.m. to 9:00 a.m. Mar. 26[1]	One pound of lean steak at	16	32	70		1.49	6.5
	9:00 a.m. to 11:00 a.m. Mar. 26	7 a.m. at rest	17	37	66		1.54	6.0
	11:00 a.m. to 1:00 p.m. Mar. 26		27	35	57		1.76	5.3
	1:00 p.m. to 3:00 p.m. Mar. 26		23	22	45		0.85	5.0
Mc.	10:00 p.m. to 7:00 a.m. Aug. 18, 19	Fasting	3	4	14	28	0.16	7.0
	7:00 a.m. to 11:00 a.m. Aug. 19	One pound of lean steak at	12	7	64	96	0.73	7.5
	11:00 a.m. to 3:00 p.m. Aug. 19	7 a.m. normal activity	13	18	51	122	0.53	6.5
Mc.	10:30 p.m. to 7:00 a.m. Aug. 22, 23	Fasting	24	28	49	92	0.59	5.3
	7:00 a.m. to 11:00 a.m. Aug. 23	One quart of milk at 7	7	13	36	80	0.80	7.5
	11:00 a.m. to 3:00 p.m. Aug. 23	a.m. normal activity	13	17	43	75	0.65	6.3
H.	7:30 a.m. to 9:30 a.m. Feb. 18	One pound of steak at	9	23	50			7.0
	9:30 a.m. to 11:30 a.m. Feb. 18	7:30 a.m. at rest	5	9	42			7.6
	11:30 a.m. to 1:30 p.m. Feb. 18		9	16	60			7.3
	1:30 p.m. to 3:30 p.m. Feb. 18		21	21	54			5.7
C.	9:00 a.m. to 11:30 a.m. Feb. 25	One pound of steak at	8	8	32			6.9
	11:30 a.m. to 1:30 p.m. Feb. 25	9 a.m. at rest	13	11	66			7.0
	1:30 p.m. to 3:30 p.m. Feb. 25		12	16	51			6.6
	3:30 p.m. to 4:30 p.m. Feb. 25		11	16	36			6.2
B.	6:00 a.m. to 8:45 a.m. Aug. 31	Fasting	21	28	42	85	0.46	5.2
	8:45 a.m. to 2:00 p.m. Aug. 31	One pound of steak at	15	25	28	120	0.66	6.0
	2:00 p.m. to 4:45 p.m. Aug. 31	8:45 a.m. normal activity	18	14	45	106	0.66	4.9

[1] Day following the ingestion of 3 pounds of steak.

for subject H. The fasting plasma bicarbonate value for subject M. was 4 volumes per cent higher following a night during which the urinary excretion was essentially acid (1361 cc. 0.1 N acid per 24 hours) than when the excretion was more nearly neutral (144 cc. 0.1 N acid per 24 hours). These results might at first sight appear to be at variance with those of Fitz and Van Slyke ('17) who found that urinary acidity as measured by titratable acidity and ammonia could be correlated with plasma CO_2 combining power, if concentration was also considered. For normal individuals the average error found by Fitz and Van Slyke was 3.2 volumes per cent, the maximum 7 volumes per cent. Van Slyke has emphasized that the correlation is only an approximation. The changes in plasma CO_2 observed in our studies were, in all instances, well within the error of the Fitz-Van Slyke determinations. Our results would indicate that the recent deductions of Saywell ('32) that raisins and figs produce a more alkaline body reaction because they produce a decrease in urinary acid are extremely hazardous. It appears to us that acid-base data for the blood should have been collected in the experiments of Saywell before deductions were drawn.

DISCUSSION

The equivalent in $NaHCO_3$ as alkaline ash of 1 pound of bananas is 2 gm. This foodstuff was included in the present studies because it does not contain the large amount of organic acid found in oranges. One quart of orange juice contains the alkaline ash equivalent of roughly 4 gm. of $NaHCO_3$. Since the enormous amounts of citric acid present must show a temporary acid effect (oxidation is not quantitative), and since 4 gm. of $NaHCO_3$ only temporarily shifts the CO_2 combining power by 2 volumes per cent, one would, on theoretical grounds, expect no significant change in the acid-base equilibrium of the blood to the alkaline side following the ingestion of orange juice or, for that matter bananas. In Blatherwick's classic experiments on the orange ('22), it was shown that the tolerance of the body to citric acid oxidation was not ex-

ceeded. Slight increases in urinary alkalinity were observed, since the base excreted overshadowed the amount of organic acid not oxidized. Blatherwick concluded, "One may therefore feel secure in eating unlimited amounts of oranges without fear of acidotic effects." He was fully aware of the very slight excess of base over acid excreted.

The experiments with orange juice, bananas, and milk, while they offered no theoretical ground for an expected change in alkali reserve, serve as a basis of comparison for the acid ash food steak. In only one of the four individuals studied could a significant temporary lowering of the plasma $BHCO_3$ be demonstrated following the ingestion of a pound of steak. The question arises as to whether this effect should be ascribed to the liberation of acid ash in the catabolism of the food or to the re-absorption of acid following the alkaline tide. The latter explanation would appear probable since on the occasion of the maximum fall of plasma bicarbonate, the excretion of urinary acid, ammonia, nitrogen, and phosphorus was decreasing. That the effect is transient is demonstrated by the high pre-breakfast value found for the same individual following a day in which 3 pounds of steak were ingested, and confirmed by the data obtained for the individual who ate the equivalent amount of acid ash food for 2 days. A calculation of the maximum rate of acid excretion by the kidneys on the steak diet, as equivalents of ammonium chloride, yields only 0.3 gm. per hour. The equivalent of 1 pound of steak as ammonium chloride is approximately 3 gm. The liberation of the acid in the catabolism of the steak obviously covers a period exceeding 6 hours, as gauged by phosphorus, sulfur, and nitrogen excretions. Barring the added effect of reabsorption of acid following the alkaline tide, slow liberation of acid in the catabolism of the steak would in itself be insufficient to cause a significant change in the body alkalinity.

The results of the study indicate rather clearly that no significant change in the reaction of the blood (pH) following the ingestion of either acid or ash or alkaline ash foods is induced. The significance of the isolated observation of a

temporary fall in the plasma bicarbonate following the acid ash food in relation to a possible physiological effect remains to be considered. The fall, 3 m.Mol., is less than that induced by exercise and was not increased when normal activity was superimposed. Moreover, the absolute values for the low bicarbonate contents are well within the normal fasting range of the other individuals studied.

SUMMARY

1. The daily administration of 30 gm. of sodium citrate was the smallest amount of alkali which produced a shift in the acid-base equilibrium of the blood drawn before breakfast, outside the normal variation of the individual, but inside the normal variation of a normal group.

2. There was no significant difference in the acid-base picture of the blood of a normal individual drawn before breakfast whether the mixed diet consumed contained excessively acid or excessively alkaline ash foodstuffs. The diet regularly consumed by the individual contained potentially as much acid ash as a diet planned to be excessively acid. A diet containing 240, 204, and 93 gm. of protein on consecutive days and 200 cc. N acid equivalent of ash daily, produced a doubtfully significant shift in the blood picture to the acid side.

3. The ingestion of 1 quart of milk, 1 quart of orange juice or 1 pound of bananas produced no temporary shift in the plasma pH or alkali reserve.

4. The ingestion of 1 pound of steak temporarily lowered the plasma bicarbonate significantly in one of four individuals studied. The absolute values were well within the group range.

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SOME FACTORS INFLUENCING NITROGEN ECONOMY DURING PREGNANCY[1]

CALLIE MAE COONS[2] AND GLADYS B. MARSHALL
Department of Agricultural Chemistry Research, Oklahoma Agricultural Experiment Station, Stillwater

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The phase of metabolism in human pregnancy which was among the earliest to be studied was that of nitrogen exchange, beginning with Zacharjewsky (1893). Since then some fifteen investigations have been reported from various laboratories and yet the information on this subject appears at times to be hopelessly confused, and our concept of the nitrogen needs of the human mother during pregnancy often seems anything but clear.

Only the recognition of the multiplicity of factors, both fetal and maternal, affecting nitrogen economy during pregnancy; the observation of nitrogen metabolism in conjunction with that of other elements, particularly phosphorus, and with a knowledge of the general adequacy of the diet as a whole, such as total calories, available vitamins and characteristic foodstuffs; together with data on larger numbers of subjects with known nutritional histories can effect an accurate solution of this problem. The material presented in this paper adds to the accumulation of evidence and serves to indicate some reasons why past findings have been so variable.

LITERATURE

Reviews of the significance of various phases of nitrogen metabolism during pregnancy are given by Harding ('25)

[1] Published with the permission of the Director of the Agricultural Experiment Station, Stillwater, Oklahoma.

[2] Mary Pemberton Nourse Memorial Fellow of the American Association of University Women, 1931–1932.

and more recently by Hunscher et al ('33). A summary of the nitrogen balance experiments during pregnancy which have been reported in the literature including the present paper have been compiled in table 1. This summary does not include the experiments by Sillevis ('04) and additional ones

TABLE 1

Summary of nitrogen balance studies during pregnancy

INVESTIGATOR AND REFERENCE	NUMBER OF SUBJECTS	NUMBER OF BALANCES	TOTAL DAYS	AVERAGE NITROGEN DAILY		COMMENT
				Intake	Storage	
				Gm.	*Gm.*	
Zacharjewsky (1893)	6	6	60	18.54	3.05	6 to 18 days antepartum. Limited diet
Schrader ('00)	2	2	11	22.37	6.36	4 to 7 days antepartum. Computed diet
Slemmons ('04)	4	4	39	14.89	2.39	2 sets twins. Diets computed
Hahl ('05)	2	7	41	18.00	2.04	17 to 24 days antepartum
Bar ('05)	3	3	34	17.43	6.68	Low fecal nitrogen
Hoffström ('10)	1	23	161	12.90	1.84	Continuous observation
Landsberg ('12)	6	13	53	16.10	2.50	
Landsberg ('14)	9	14	70	16.10	1.23	
Wilson ('16)	3	37	259	13.14	2.84	Continuous observation. Computed diets
Sandiford et al. ('31)	1	5	50	13.88	0.30	Computed diets. Feces not analyzed
Macy et al. ('31)	1	2	15	11.80	2.55	Specified diet
Hunscher et al. ('33)	3	12	49	17.58	1.90	Pregnancy subsequent to heavy lactation
Coons-Blunt ('30)	9	23	116	11.03	2.15	Home diets
This paper	6	23	92	11.58	1.97	Home diets

from Bar ('07), for which the figures at the authors' disposal were only partially complete. The summary shows the number of observations and subjects and the average daily intake and storage found by the various investigators. All of these results are far from being comparable, but some of the differentiating characteristics have been listed under comments.

The workers prior to Hoffström concerned themselves with studies during the very last days of pregnancy and often used subjects requiring hospitalization for one reason or another. The case histories are often brief or entirely lacking. There is no record of a preliminary period or of only very short ones for accustoming the subjects to the changes in diet. Usually the diet was limited as to variety, and in one case it consisted of only milk, meat, bread, tea, and water (Zacharjewsky, 1893).

Another important source of experimental error, which extends also to some later studies, was the use of published analyses for computing the nitrogen content of the diets. Donelson and co-workers ('31) have shown that errors from this procedure may vary from 1 to 38 per cent, being more significant in the short time studies and arising apparently from differences in composition of the foodstuffs rather than from errors in calculation. Thus an error of only 5 per cent in the computation of a high protein diet would change the balance approximately 1 gm., representing from 30 to 100 per cent of the amount stored in most cases.

Records of very low fecal nitrogen, 0.2 to 0.5 gm. daily in some studies (Bar, '07; Slemmons, '04; Wilson, '16) indicate coefficients of digestibility for nitrogen around 95 to 99. Such figures suggest the possibility of improper preservation of collections, which, if true for urine, might easily account for very high retentions through losses of urinary nitrogen. Even the error from failure to analyze feces may equal or exceed a small balance, since some data (Coons, '30) show coefficients of digestibility as low as 70, leaving three times as much fecal nitrogen as the customary coefficient of 90 would allow. The entire series of twenty-three balances for nine women reported previously by one of us (table 3) showed an average coefficient of 83 or almost twice the expected amount of fecal nitrogen.

The chief difficulty in the way of comparing these nitrogen balances is the wide variation between the nitrogen consumption, around 18 to 20 gm. daily, of the subjects of the early

studies, and the 11 to 12 gm. daily of those of the more recent series. This confirms previous observations made in this laboratory (Coons, '32) that there is a tendency nowadays to habitually lower protein consumption, and finds support in the lower urinary nitrogen figures reported by Rowe and his associates ('30). It seems probable also that more of the nitrogen of these recent lower diets comes from vegetable or cereal proteins, and less of it from animal sources.

Exceptions to these records of low nitrogen consumption are two of Macy's women who had become accustomed to high intakes, as high as 28 to 29 gm. of nitrogen daily, in their performance of heavy lactation and had carried over some of this tendency into the subsequent gestation period.

On the whole, those studies (table 1) which cover most of pregnancy, namely Hoffström's work and all later ones, representing a total of thirty-nine women, showed a definite tendency to an average storage around 2 gm. of nitrogen daily. There is no evidence in the data available to indicate that retentions are at all constant throughout the whole of pregnancy, because actually wide and irregular fluctuations in storage occur even when the same individual is observed continuously for only a part of gestation. However, cause for individual retentions varying greatly from the above average should be sought in the quality and quantity of the diet, in the previous nutritional history of the mother during both the gravid and non-gravid states, or in some other unusual phenomena associated with the case.

EXPERIMENTAL

The data herein reported included twenty-three 4-day balance experiments during pregnancy and one 4 weeks prior to pregnancy on six women. The activities of these women have been described (unpublished data) and more complete case histories, including total weight changes, age, size of the baby, and success in lactation, are presented elsewhere (Coons, '33).

The manner of weighing the food and making collections was the same as that used in previous studies (Coons and

Blunt, '30; Coons, '30). A portable refrigerator was devised and used throughout the series as an extra precaution for preservation of excreta from the time of collection until removal from the home to the laboratory, since many of the metabolism periods were scheduled for warm months in a southern climate. This apparatus consisted of a double compartment, well-insulated fireless cooker, placed in the home, usually on a back porch, and cooled with ice cubes from an electric refrigerator. Rubber ice bags were used to hold the cubes and these were refilled by the investigator two to three times daily as needed. In this manner feces and urine were cooled rapidly from body temperature to about 40° to 50°F., remaining at this temperature until the next visit by the investigator when collections were removed to the laboratory and cared for at once. As customary in this work, toluol was also used in the bottles for urine collections.

Determinations of creatinine daily left no doubt as to the completeness of the collections of urine. Total nitrogen was determined daily on urine and also on the composite aliquot made up for mineral analyses. Analyses were made on the dried samples of food and feces, composited for the period. The macro-Kjeldahl method was employed throughout for determining total nitrogen.

RESULTS AND DISCUSSION

The nitrogen balances are shown in table 2, and table 3 summarizes some of the dietary habits in relation to the absorption of food.

From table 2 it will be seen that the intake varied widely, 8.08 to 19.13 gm. daily, and that although the retention varied somewhat, in fourteen instances it was between 1 and 2 gm. daily. Analyses for total energy content of the diet (Coons, '33) showed a range in intake from 36 to 58 calories per kilogram with an average of 44, and indicated no marked dietary deficiency in this respect. Differences in retention rates were apparently unrelated to any period of gestation, and tendencies to high storage were individualistic, exhibited only by cases II and VI.

The higher retentions of these cases were associated with higher levels of intake, a relatively larger proportion of milk, meat, and eggs in the diet (table 3) and larger amounts of the vitamin B complex. The extra supply of this last factor came from an ounce of Vitavose daily, the whole grain cereals

TABLE 2

Nitrogen retention by Oklahoma women during pregnancy

CASE AND PERIOD		WEEK OF PREGNANCY	NITROGEN INTAKE	NITROGEN OUTGO			BALANCE	NITROGEN PER KILO	
				Urine	Feces	Total		Intake	Retained
			Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
I	0	− 4	8.16	6.82	0.85	7.67	+ 0.49	0.17	0.010
	1	2	9.37	6.79	0.88	7.67	+ 1.70	0.19	0.030
	2	27	9.61	7.40	1.04	8.44	+ 1.17	0.17	0.021
	3	31	9.89	7.44	1.00	8.74	+ 1.15	0.17	0.019
	4	34	9.88	7.83	1.04	8.88	+ 1.00	0.16	0.016
	5	38	8.80	5.98	0.91	6.89	+ 1.91	0.14	0.030
II	1	26	11.84	8.13	1.26	9.39	+ 2.44	0.22	0.045
	2	30	14.26	9.13	1.35	10.48	+ 3.78	0.25	0.067
	3	34	14.62	9.92	1.60	11.52	+ 3.10	0.25	0.052
III	1	33	11.56	8.57	1.58	10.15	+ 1.40	0.16	0.020
	2	37	12.86	8.85	1.75	10.60	+ 2.26	0.18	0.031
IV	1	31	11.84	9.18	1.22	10.40	+ 1.44	0.14	0.017
	2	35	9.74	7.18	1.27	8.45	+ 1.29	0.11	0.015
	3	39	11.64	8.64	1.45	10.09	+ 1.56	0.13	0.017
V	1	28	10.08	7.38	1.02	8.40	+ 1.68	0.16	0.026
	2	33	8.08	6.02	1.33	7.35	+ 0.73	0.12	0.011
	3	38	10.01	5.85	1.34	7.19	+ 2.82	0.15	0.043
	4	39	8.97	6.61	1.25	7.86	+ 1.11	0.14	0.017
VI	1	18	17.70	12.46	2.07	14.53	+ 3.17	0.32	0.058
	2	23	19.13	11.99	1.94	13.93	+ 5.20	0.32	0.087
	3	27	13.54	10.59	1.50	12.09	+ 1.45	0.22	0.023
	4	31	12.01	8.12	2.04	10.16	+ 1.85	0.18	0.028
	5	35	11.96	7.64	1.61	9.25	+ 2.71	0.18	0.041
	6	38	9.90	7.31	1.57	8.85	+ 1.02	0.15	0.015

and liver regularly (unpublished data) for case II, and for case VI in the first two periods from much milk, liver, and whole grain cereals, the last two of which did not appear in the diets of the later studies with her. Experimental evidence of the relation of the vitamin B complex to nitrogen economy

has been presented by various investigators (Funk, '23; Macy et al., '30; Sherman and Derbigny, '32). It is impossible, however, from these experiments on freely self-chosen diets

TABLE 3

The character of the diet as related to the absorption of nitrogen

CASE AND PERIOD		WEIGHT	CHIEF PROTEIN-CONTAINING FOODS AVERAGE DAILY INTAKE				ABSORPTION			
			Meats	Liver	Eggs	Milk	Nitrogen coeff. of digestibility	Food daily (dry)	Feces daily (dry)	Food: feces
		Kilo	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent*	*Gm.*	*Gm.*	
I	0[1]	49.0					90	355	17	20
	1[1]	49.9					91	433	17	25
	2	56.7	4	9	36	697	89	433	24	18
	3	59.0	31		39	536	90	461	21	22
	4	61.2	30		30	600	90	439	23	19
	5	63.5	21		22	527	90	482	21	23
II	1	54.4	34	17	56	593	89	538	26	20
	2	56.2	78	12	68	835	91	560	26	21
	3	59.4	62	19	61	726	89	560	31	18
III	1	71.2	144		42	178	86	518	36	14
	2	72.5	43		38	459	86	627	28	22
IV	1	86.6	115		11	329	90	575	27	21
	2	88.4	20		11	465	86	541	25	22
	3	90.7	46			649	88	705	31	23
V	1	63.5	30		60	209	90	499	18	28
	2	64.4	11		58	195	84	454	27	17
	3	65.3	57		19	249	87	519	26	20
	4	65.8	24		22	485	86	501	25	20
VI	1	54.9	30	48	68	1162	88	577	41	14
	2	60.0	36	21	50	1285	90	617	33	18
	3	62.6	31		44	796	89	542	26	21
	4	65.3	10		31	1008	83	553	39	14
	5	65.8			10	887	87	492	33	15
	6	66.2				957	84	465	29	16
Average:										
This series			39	21	35	628	88	530	28	20
Chicago series			33	21	29	416	83	464	34	14

[1] Record of foodstuffs not retained.

to estimate the relative importance of these dietary factors in their effect on nitrogen economy. Besides these, too many non-dietary influences were playing a part.

The gains in weight of the different subjects during the time covered by these balance studies were similar, around 2 kg. a month, except in cases III and V and in case VI during the last 2 months (table 2). However, there was no reason to believe that such gains were of similar body composition. The total gains from the prepregnant weight to that at the end of gestation showed greater differences in individuals and were more indicative of changes in nutritional status in the maternal organism itself.

For example, case IV, a tripara, some 30 pounds overweight according to commonly used standards, gained only 15 pounds during gestation and that during the last trimester. Case VI, some 20 pounds below weight at the beginning, gained a total of 35 pounds, 30 of it before the thirtieth week of pregnancy. If it be assumed that, in the instance of case VI, the maternal organism brought itself to a state of good nutrition during gestation, as indeed outward appearances and a feeling of well-being indicated, then a gain of only 15 pounds remained for the pregnancy proper. In like manner it was found that the other primiparae, cases I, II, and V, gained 15 to 20 pounds in excess of that needed for normal nutrition.

The fact that if excessive gains occurred they did so before the last trimester of pregnancy, the period for greatest fetal accretions, and evidence here and elsewhere (Coons and Blunt, '30; Hunscher et al., '33) that very high nitrogen retentions, when found, tended to occur early in pregnancy, emphasize the need for a knowledge of the nutritional history of the mother during all of gestation as well as during the pregestation period, in order to interpret the findings for nitrogen retention. In other words, it is futile to expect a given level or curve of nitrogen retention for all women during the course of a pregnancy, unless a normal, well-nourished maternal organism at the beginning, and the same state of nutrition without addition or subtraction at the end of gestation, is presupposed.

In the previous study lactation seemed related to nitrogen metabolism (Coons and Blunt, '30). It is of interest to add

here that in this series, cases III, IV, and VI were quite successful, case II had difficulty with inverted nipples, and case I, threatening failure at first, finally, after some 4 weeks with special attention to diet, rest and avoidance of worry, established full breast feeding which continued satisfactorily until weaning. On the other hand, case V failed completely, and the infant was receiving all artificial feedings by the sixth week. Both the diets and storages of nitrogen for cases I and V were the poorest of the series and are believed to represent borderline deficiencies for women of their nutritional status.

Attention is called to another interesting observation in this series of studies. From tables 1 and 3 it will be seen that the diets averaged slightly better in quantity and quality than did those of the Chicago series representing similar home conditions and technic of investigation. Despite an apparently poorer absorption in the earlier group the storage was slightly higher, averaging 2.15 compared to 1.97 gm. daily, or 19.5 to 17.0 per cent of the respective intakes. Omitting the storage figures for those periods when the diets contained unusual amounts of the vitamin B complex (case II) or were much above the customary intake (case VI, periods 1 and 2), the present group averaged a storage of only 1.57 gm. daily, compared to the 2.15 gm. for the northern group with similar nitrogen consumption.

A poorer absorption was shown for the Chicago group by the coefficients of digestibility for nitrogen, with the average of 83 equal to the lowest coefficient recorded in the present series, and likewise the average of 88 for this group equal to the highest recorded in the former study. It was shown also by the ratio of dry food to dry feces daily, representing crudely the high or low absorption of all foodstuffs, which ratios averaged 20 and 14 for the present and former series respectively. The diets of the southern group were characterized by larger amounts of vegetables and cereals (Coons, '33), as was indicated also by the greater dry weight for food composites daily (table 3). The more frequent use of laxa-

tives by these women was explained partly by these tendencies to lower fecal residues. Such tendencies were coincident with better calcium retention (unpublished data) and may have been referable to the increased sunlight. These facts serve to accent the poorer nitrogen retention in these Oklahoma women and point to some disturbed endogenous metabolism. The disturbance may have been due to a low quality of protein or a lack of some accessory factor and may be related to the lower basal metabolism reported for this section. At least it calls for further investigation.

In summarizing it would seem, however, that all the possible factors influencing nitrogen metabolism tend to operate during pregnancy. On the positive side are the impulses to replenish body reserves, to supply elements to a growing organism, and to provide for some vaguely defined but quantitatively large biological preparation for the losses of parturition and the puerperium and for the performance of lactation. Along with these come the increased desire for food, and consequent higher intake, the improved utilization, and the tendency to retain that absorbed or economize on protein catabolism, all a part of the normal events in any pregnancy. On the negative side, however, there may be operating one or more of such factors as the poor quality or limited quantity of protein available, a series of digestive disturbances such as nausea and 'heart-burn' common to pregnancy, or other pathological conditions necessitating a restriction of the amount of protein available for absorption. Doutbless other unknown conditions affect one or more phases of the cycle of protein metabolism. It is surprising that the maternal organism adjusts itself as well as it does to all these varied influences.

SUMMARY

Twenty-three 4-day balance experiments during pregnancy and one 4 weeks prior to pregnancy on six women are presented in this report.

The results show relatively lower nitrogen retentions than those of previous studies, except when the diets were increased with additional protein or supplemented with accessory factors, particularly the vitamin B complex. The average intake, including supplemented diets, was 11.58 gm. of nitrogen daily with a storage of 1.97 gm.

Tendencies to greater absorption rates as shown by higher coefficients of digestibility and by lower fecal residues were observed in this series and are discussed in relation to the metabolism of other elements.

A knowledge of the nutritional history of the mother, both in the pregravid period and through all of gravidity is essential to the proper interpretation of nitrogen retentions during pregnancy.

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THE IRON CONTENT OF FOODS USED IN A MUNICIPAL HOSPITAL

VINCENT TOSCANI AND PAUL REZNIKOFF

Russell Sage Institute of Pathology in affiliation with the Second Medical (Cornell) Division of Bellevue Hospital and Department of Pathology, Bellevue Hospital, New York

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In the course of a study of iron metabolism, in the metabolic ward of the Russell Sage Institute of Pathology, analyses of the iron content of the food, provided by the diet kitchen of Bellevue Hospital, were made throughout the experiment. Because of the fact that this food is representative of the diet served patients in a large municipal hospital, it is thought desirable to present the results obtained.

The specimens of food were collected over a period of 7 months and, with the exception of fruits contained in jars and fresh peaches, at least three samples of each specimen were studied. No two samples of the same food were analyzed at the same time. The subjects receiving the foods were allowed only distilled water or beverages prepared with distilled water. All their food was cooked in iron-free water. No iron pots or pans were used. The foods were prepared in enamel kitchenware and with heavily plated cutlery. The specimens for analysis were sent to the laboratory in glass bottles. All of the fresh vegetables were thoroughly washed with iron-free water before drying to insure freedom from soil. Foods which contained considerable moisture were dried in 100 gm. portions in porcelain casseroles on a steam bath. The dried foods were first weighed, then thoroughly ground in a porcelain mortar, passed through a fine copper mesh sieve and an aliquot portion was taken for analysis. The opening

TABLE 1

The iron content of foods (milligrams per 100 gm. E.P.) compared to values given in standard tables

NO.	FOOD	NUMBER OF SPECIMENS	MAXIMUM	MINIMUM	AVERAGE	P & E ('28)	SHERMAN ('32)	ROSE ('29)	OTHER SOURCES
1	Apples, f	3	0.31	0.26	0.29	0.31	0.36	0.30	
2	Apricots, c.	3	1.02	0.68	0.86		0.60		
	Apricots, d.	3	7.25	6.45	6.74	7.26		1.40	
3	Asparagus, c.	3	1.20	0.44	0.73	0.79	1.00 (f.)	1.00 (f.)	1.70 (f.) L,C&A
4	Bacon, f.	3	0.94	0.67	0.80		1.50	1.30	
5	Bananas, f.	3	0.60	0.35	0.46	1.76	0.64	0.60	
6	Beans, lima, c.	3	2.20	2.09	2.16	11.66 (f.)	2.40 (f.)	2.00 (f.) 7.00 (d.)	
7	Beans, string, c.	3	1.20	0.80	0.96				
	Beans, string, f.	3	1.05	0.63	0.91	0.93	1.00	1.10	0.38 L&F
8	Beef, lean	3	2.70	1.83	2.22		3.00	2.40	3.33 L&F
9	Bread, white	3	1.38	1.18	1.27		0.90	0.85	0.67 L&F
	Bread, whole wheat	3	1.97	1.88	1.93		1.60	1.65	
10	Butter, f.	3	0.26	0.17	0.20		0.20	0.20	
11	Cabbage, f.	3	0.64	0.38	0.53	0.34	0.43	1.10	
12	Carrots, f.	3	0.64	0.43	0.54	1.07	0.64	0.60	0.51 L&F
13	Cauliflower, f.	3	1.09	0.95	1.02	1.43	0.94	0.60	
14	Celery, f.	3	0.80	0.35	0.51	0.77	0.62	0.50	
15	Cheese, American	3	0.89	0.64	0.79	1.38	1.30	1.30	
16	Cherries, c.	3	1.38	0.93	1.18	0.48 (f.)	0.40 (f.)	0.40 (f.)	
	Cherries, jar	1			0.81				
17	Chicken, boiled	3	2.73	2.34	2.51	1.01 (d.) 0.70 (l.)		3.20	
18	Cocoa, A.P.	6	19.00	8.00	12.64	3.13	2.70	2.70	
	Cocoa, bean without shell	3	3.55	3.33	3.43				
	Cocoa, shell alone	3	57.10	22.86	35.90				
19	Coffee, whole bean	3	7.02	6.16	6.66				
	Coffee, ext. from 100 gm.	3	1.13	0.79	0.91				
20	Corn, c.	3	0.57	0.35	0.43	2.60 (f.)	0.47 (s.)	0.80 (g.)	
21	Corn flakes	5	4.92	1.64	2.98	2.78	2.90 (sd.)	0.90 (meal)	
22	Cranberries, f.	3	0.47	0.40	0.45		0.44	0.60	
23	Cream, 20 per cent	3	0.33	0.15	0.23		0.22	0.20	
24	Egg, whole	3	2.90	2.03	2.38	2.52	3.00	3.00	1.92 cooked L&F
25	Egg yolk	3	6.86	6.35	6.52	7.60	8.60	8.60	
26	Farina	3	1.23	0.89	1.02		0.80	0.80 (l.)	
27	Fish, salmon, c.	3	0.99	0.80	0.87	0.83 (f.)		1.20 (f.)	
28	Fish, tuna, c.	3	2.12	1.50	1.80			1.20 (1.4 in oil)	
29	Flour, white	3	2.00	1.33	1.73	0.91 (p.)	1.00	1.00	
30	Grapes, Malaga, f.	3	0.71	0.54	0.64	2.28	0.73	0.30	
31	Grapefruit, f.	3	0.28	0.21	0.24	0.27	0.27	0.30	
32	Lamb, f.	3	2.62	2.38	2.51			3.30 (chops)	

29	Flour, white	3	2.00	1.33	1.73	0.91 (p.)	1.00	1.00	
30	Grapes, Malaga, f.	3	0.71	0.54	0.64	2.28	0.73	0.30	
31	Grapefruit, f.	3	0.28	0.21	0.24	0.27	0.27	0.30	
32	Lamb, f.	3	2.62	2.38	2.51			3.30 (chops) 1.50 (leg med. fat)	
33	Lettuce, heart	4	0.60	0.32	0.50	0.42	0.70	0.70	0.44 L&F
	Lettuce, leaf	3	1.28	0.90	1.04	1.87			2.37 L,C&A
34	Liver, f.	3	6.17	5.07	5.66			8.10	
35	Macaroni	6	1.82	1.21	1.45		1.20	1.20	1.80 L&F
36	Milk, f.	3	0.26	0.13	0.21	0.24	0.24	0.20	
37	Oatmeal	5	6.00	4.44	5.10	3.80	3.80	3.80	
38	Olive oil	3	0.30	0.10	0.18				
39	Onions, f.	3	0.45	0.43	0.44	0.30	0.48	0.60	
40	Orange juice	3	0.36	0.20	0.27	0.28	0.24	0.20	0.19 L&F
	Orange, whole	3	0.60	0.26	0.39	0.66	0.52	0.20	
41	Peaches, c.	5	1.53	0.40	1.08				0.25 L&F
	Peaches, fresh	2	0.56	0.52	0.54	0.36	0.33	0.30	
	Peaches, jar	1			0.96				
42	Pears. c.	3	0.79	0.45	0.65				
	Pears, f.	3	0.37	0.24	0.32	0.46	0.32	0.30	
	Pears, j.	1			0.37				
43	Peas, c	3	0.95	0.81	0.89				1.23 L&F
	Peas, f.	3	1.68	1.27	1.44	1.77	2.07	1.70	
44	Peppers, green	3	1.05	0.40	0.69	0.41	0.40	0.40	
45	Pineapple, c.	3	1.66	1.21	1.39				
	Pineapple, f.	3	0.36	0.25	0.32		0.37	0.50	
	Pineapple, j.	1			0.53				
46	Potatoes, f.	3	0.73	0.65	0.69	0.85	0.91	1.30	0.46 L&F
47	Prunes, d.	3	4.65	2.19	3.57	5.17	2.85	3.00	2.03 L&F
48	Rice, white	3	1.16	0.85	0.99	1.05	0.90	0.90	
49	Salt, table	3	0.16	0.08	0.11				
50	Spinach, f.	4	5.79	3.48	4.40		2.55	3.60	3.24 L,C&A
51	Sugar, granulated	3	0.10	0.06	0.09				
52	Tea leaves, whole	3	16.32	14.05	15.46				
	Tea ext. from 100 gm.	3	0.95	0.55	0.72				
53	Tomatoes, c.	3	1.45	0.69	1.08	1.30			0.16 L&F
	Tomatoes, f.	3	0.49	0.36	0.44	0.60	0.44	0.40	0.75 L,C&A
54	Turnips. f.	3	0.56	0.42	0.49	0.70	0.52	0.50	
55	Walnuts	3	4.44	3.00	3.81	5.98 (b.) 2.14 (e.)	2.10	2.10	
56	Yeast, d.	3	15.80	13.60	14.56				

c. = canned. l. = light p. = patent. LC&A = Levine, Culp & Anderson ('32).
f. = fresh. g. = green e. = English. L&F = Leichsering & Flor ('32).
d. = dried. s. = sweet. b. = black. P&E = Peterson & Elvehjem ('28).
A.P. = as purchased. sd. = sweet dried.

of the cans, in the case of tinned food, presented a source of iron contamination. This was overcome by carefully washing the foods with distilled water as they were removed from the tins. Whenever washing was not permissible, as in the case of canned corn and fish, a specimen was collected from the center of the food, using a silver spoon to scoop out enough material for duplicate analyses, avoiding the food around the edges of the can. The canned fruits, however, were very difficult to dry, due to the large amounts of sugar present. The question of getting a representative aliquot portion was surmounted by using practically all of the partially dried fruit for ashing. Cereals, dried fruits and materials low in water content were ashed directly in porcelain crucibles.

In all cases where appreciable differences were obtained for a particular foodstuff from those given in standard tables or from those found upon previous examination, duplicate analyses were made. In all such tests good checks were obtained. The method for the determination of iron was the Elvehjem ('30) modification of the Kennedy ('27) procedure.

The results of the analyses and comparisons with values in some standard tables are given in table 1. Certain facts are apparent from these figures. In the first place, some of the foods served in this municipal hospital dietary vary rather markedly in iron content from those of the standard tables of Sherman ('32), Rose ('29), Leichsenring and Flor ('32), and Levine and co-workers ('32). The most marked differences are: bacon [0.8],[1] lean beaf [2.2], chicken [2.5], cocoa [12.6], whole egg [2.3], egg yolk [6.0], farina [1.02], tuna fish[1.9], flour [1.7], liver [5.6], oatmeal [5.1], fresh peas [1.4], green peppers [0.69], potatoes [0.68], dried prunes [3.57], fresh spinach [4.4], walnuts [3.8]. Of these seventeen items all except three are in the high iron-containing class; four are meats and four fresh vegetables.

Those foods which agree closely in iron content with the standard tables are: fresh string beans [0.96], butter [0.204],

[1] The values given in brackets are those found in our determinations in milligram Fe per 100 gm. of edible portion.

corn flakes [2.98], cranberries [0.448], cream [0.233], grapefruit [0.24], milk [0.21], fresh pears [0.32], rice [0.987], fresh tomatoes [0.443], fresh turnips [0.49]. Of these eleven foods one is a high iron-containing food, two contain a moderate amount of iron and the rest are low in iron content. Three

TABLE 2

The variation of maximum values from minimum values of iron content of different specimens of the same foods

WITHIN 25 PER CENT	26 TO 50 PER CENT	51 TO 100 PER CENT	100 PER CENT OR MORE
Apples, fresh	Bacon, fresh	Apricots, c.	Asparagus, c.
Apricots, dried	Beans, string, c.	Bananas, f.	Celery, f.
Beans, lima, c.	Beef, lean	Beans, string, f.	Cocoa, A.P.
Bread, white	Carrots, f.	Butter, f.	Cocoa shell alone
Bread, whole wheat	Cheese, American	Cabbage, f.	Corn flakes
Cauliflower, f.	Cherries, c.	Corn, c.	Cream, 20 per cent
Chicken, boiled	Coffee percolated from 100 gm.	Flour, white	Milk, f.
Cocoa bean without shell	Egg, whole	Lettuce, heart	Olive oil
Coffee, whole	Farina	Macaroni	Orange, whole
Cranberries, f.	Fish, tuna, c.	Orange juice	Peaches, c.
Egg, yolk	Grapes, Malaga, f.	Pears, c.	Peppers, green
Fish, salmon, c.	Grapefruit, f.	Pears, f.	Prunes, dried
Lamb, f.	Lettuce, leaf	Spinach, f.	Salt, table
Liver, f.	Oatmeal	Sugar, granulated	Tomatoes, c.
Onions, f.	Peas, f.	Tea, extract from 100 gm.	
Peaches, f	Pineapple, c.		
Peas, c.	Pineapple, f.		
Potatoes, f.	Rice, white		
Tea leaf, whole	Tomatoes, f.		
Yeast, dried	Turnips, f.		
	Walnuts		

of the foods which approximate in iron value those given in standard tables are fresh vegetables, two are fruits (fresh), and three are milk products.

It is of interest to note that of the thirteen canned foods examined, cherries [1.183], tuna fish [1.797], peaches [1.08], pears [0.653], pineapple [1.39], tomatoes [1.08], six in all, have a much higher iron content than they show in the fresh

state. Single determinations of four fruits in jars, cherries, peaches, pineapple and pears were made, and in each case iron values were obtained which were higher than those in the fresh state, but lower than in the canned condition.

Table 2 illustrates the variation in iron values of the same food when analyses are made on portions taken from supplies given to the patients after an interim of several months. Twenty foods show less than 25 per cent variation between maximum and minimum values; twenty-one between 26 and 50 per cent; fifteen between 51 and 100 per cent, and fourteen more than 100 per cent.

There is no special class of food which shows any marked consistency in the relationship between maximum and minimum values except possibly meats, fish and eggs in which less variation is obtained than in the case of milk, cream and butter.

One of the important factors to be considered in arranging a diet is not so much the number of milligrams of iron in 100 gm. of the edible portion of food, but the actual amount of iron ingested when the weight of an average portion is taken into consideration. Table 3 shows the absolute quantity of iron in average portions and table 4 gives a list of foods arranged in order of iron content per 100 gm. of edible portion and in order of iron content per average portion. When the weight of an average portion of food is taken into consideration, such high iron-containing foods as cocoa, walnuts, corn flakes, flour, macaroni and lettuce leaf afford relatively little iron in the ordinary diet.

CONCLUSIONS

1. Two hundred twenty-six samples of fifty-five common foodstuffs used in a municipal hospital were analyzed for iron content and considerable variation was found in different samples of the same food.

2. Seven of thirteen canned foods which were analyzed contained more iron than in the fresh state, probably due to concentration in the process of canning and possibly due to iron contamination from the can.

TABLE 3

Actual average iron intake (weight of average portion multiplied by per cent of iron)

FOOD	WEIGHT OF AVERAGE PORTION IN GRAMS	AVERAGE IRON CONTENT MILLIGRAM PER 100 GM.	TOTAL IRON PER AVERAGE PORTION IN MILLIGRAM
Apples, f.	110	0.29	0.36
Apricots, dried	40	6.74	2.70
Apricots, c.	100	0.86	0.86
Asparagus, c.	100	0.73	0.73
Bacon, f.	40	0.80	0.32
Bananas, f.	100	0.46	0.46
Beans, lima, c.	50	2.16	1.08
Beans, string, c.	100	0.96	0.96
Beans, string, f.	100	0.91	0.91
Beef, lean	80	2.22	1.72
Bread, white	65	1.27	0.76
Bread, whole wheat	65	1.93	1.16
Butter	30	0.20	0.06
Cabbage, f.	100	0.53	0.53
Carrots	100	0.54	0.54
Cauliflower, f.	125	1.02	1.28
Celery, f.	50	0.51	0.26
Cheese, American	25	0.79	0.20
Cherries, c.	100	1.18	1.18
Chicken, boiled	100	2.51	2.51
Cocoa, ground, A.P.	5	12.64	0.63
Coffee, percolated from 12.5 gm.	12.5	0.91	0.11
Corn, c.	100	0.43	0.43
Corn flakes	25	2.98	0.75
Cranberries, f.	30	0.45	0.13
Cream, 20 per cent	30	0.23	0.07
Egg, whole	60	2.38	1.43
Egg yolk	30	6.52	1.96
Farina	25	1.02	0.26
Fish, salmon, c.	65	0.87	0.57
Fish, tuna, c.	100	1.80	1.80
Flour, white	5	1.73	0.09
Grapes, Malaga, f.	100	0.64	0.64
Grapefruit, f.	100	0.24	0.24
Lamb, f.	100	2.51	2.51
Lettuce, heart	35	0.50	0.18
Lettuce, leaf	35	1.04	0.36
Liver, f.	80	5.66	4.53
Macaroni	60	1.45	0.87
Milk, f.	200	0.21	0.42
Oatmeal	30	5.10	1.53
Olive oil	5	0.18	0.01
Onions, f.	100	0.44	0.44
Orange juice	100	0.27	0.27
Orange, whole	100	0.39	0.39
Peaches, c.	100	1.08	1.08
Peaches, f.	100	0.54	0.54
Pears, f.	110	0.32	0.36
Pears, c.	100	0.65	0.65
Peas, c.	100	0.89	0.89
Peas, f.	100	1.44	1.44
Peppers, green	100	0.69	0.69
Pineapple, f.	100	0.32	0.32
Pineapple, c.	100	1.39	1.39
Potatoes	100	0.69	0.69
Prunes, dried	50	3.57	1.79
Rice	45	0.99	0.44
Spinach, f.	100	4.40	4.40
Sugar, granulated	35	0.09	0.03
Tea extract from 4 gm.	4	0.72	0.03
Tomatoes, f.	100	0.44	0.44
Tomatoes, c.	150	1.08	1.62
Turnips, f.	100	0.49	0.49
Walnuts	10	3.81	0.38

TABLE 4

FOODS ARRANGED ACCORDING TO ABSOLUTE IRON CONTENT (MILLIGRAMS PER 100 GM. E.P.)		FOODS ARRANGED ACCORDING TO IRON VALUES WHEN AVERAGE INTAKE OF PORTION IS CONSIDERED (MILLIGRAMS)	
Cocoa shell alone	35.90	Liver, fresh	4.53
Tea leaf, whole	15.46	Spinach, fresh	4.40
Yeast, dried	14.56	Apricots, dried	2.70
Cocoa, A.P.	12.64	Chicken, boiled	2.51
Apricots, dried	6.74	Lamb, fresh	2.51
Coffee, whole, ground	6.66	Egg yolk	1.96
Egg yolk	6.52	Fish, tuna, canned	1.80
Liver, fresh	5.66	Prunes, dried	1.79
Oatmeal	5.10	Beef, lean	1.72
Spinach, fresh	4.40	Tomatoes, canned	1.62
Walnuts	3.81	Oatmeal	1.53
Prunes, dried	3.57	Peas, fresh	1.44
Cocoa bean without shell	3.43	Egg, whole	1.43
Corn flakes	2.98	Pineapple, canned	1.39
Chicken, boiled	2.51	Cauliflower, fresh	1.28
Lamb, fresh	2.51	Cherries, canned	1.18
Egg, whole	2.38	Bread, whole wheat	1.16
Beef, lean	2.22	Beans, lima, canned	1.08
Beans, lima, canned	2.16	Peaches, canned	1.08
Bread, whole wheat	1.93	Beans, string, canned	0.96
Fish, tuna, canned	1.80	Beans, string, fresh	0.91
Flour, white	1.73	Peas, canned	0.89
Macaroni	1.45	Macaroni	0.87
Peas, fresh	1.44	Apricots, canned	0.86
Pineapple, canned	1.39	Bread, white	0.76
Bread, white	1.27	Corn flakes	0.75
Cherries, canned	1.18	Asparagus, canned	0.73
Peaches, canned	1.08	Peppers, green	0.69
Tomatoes, canned	1.08	Potatoes	0.69
Lettuce, leaf	1.04	Pears, canned	0.65
Cauliflower, fresh	1.02	Grapes, Malaga, fresh	0.64
Farina	1.02	Cocoa, A.P.	0.63
Rice, white	0.99	Fish, salmon, canned	0.57
Beans, string, canned	0.96	Peaches, fresh	0.54
Coffee percolated from 100 gm.	0.96	Carrots, fresh	0.54
Peaches, jar	0.96	Cabbage, fresh	0.53
Beans, string, fresh	0.91	Turnips, fresh	0.49
Peas, canned	0.89	Bananas, fresh	0.46
Fish, salmon, canned	0.87	Rice, white	0.44
Apricots, canned	0.86	Onion, fresh	0.44
Cherries, jar	0.81	Tomatoes, fresh	0.44
Bacon, fresh	0.80	Corn, canned	0.43
Cheese, American	0.79	Milk	0.42
Asparagus, canned	0.73	Orange, whole	0.39
Tea, extract from 100 gm.	0.72	Walnuts	0.38
Peppers, green	0.69	Lettuce, leaf	0.36
Potatoes, fresh	0.69	Pears, fresh	0.36
Pears, canned	0.65	Apples, fresh	0.36
Grapes, Malaga, fresh	0.64	Pineapples, fresh	0.32
Peaches, fresh	0.54	Bacon, fresh	0.32
Carrots, fresh	0.54	Orange juice	0.27
Pineapple, jar	0.53	Celery, fresh	0.26
Cabbage, fresh	0.53	Farina	0.26
Celery, fresh	0.51	Grapefruit, fresh	0.24
Lettuce, heart	0.50	Lettuce, heart	0.18

TABLE 4—*Continued*

FOODS ARRANGED ACCORDING TO ABSOLUTE IRON CONTENT (MILLIGRAMS PER 100 GM. E.P.)		FOODS ARRANGED ACCORDING TO IRON VALUES WHEN AVERAGE INTAKE OF PORTION IS CONSIDERED (MILLIGRAMS)	
Turnips, fresh	0.49	Cheese, American	0.17
Bananas, fresh	0.46	Cranberries, fresh	0.13
Cranberries, fresh	0.45	Coffee, extract from 12.5 gm.	0.11
Onions, fresh	0.44	Flour, white	0.09
Tomatoes, fresh	0.44	Cream, 20 per cent	0.07
Corn, canned	0.43	Butter	0.06
Orange, whole	0.39	Sugar	0.03
Pears, jar	0.37	Tea, extract from 4 gm.	0.03
Pineapple, fresh	0.32	Olive oil	0.01
Pears, fresh	0.32		
Apples, fresh	0.29		
Orange juice	0.27		
Grapefruit, fresh	0.24		
Cream, 20 per cent	0.23		
Milk, fresh	0.21		
Butter, fresh	0.20		
Olive oil	0.18		
Salt, table	0.11		
Sugar, granulated	0.09		

3. In determining the iron content of diets it is important to take into consideration the weight of an average portion of food as well as the percentage of iron.

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PROTEIN, CALCIUM AND PHOSPHORUS INTAKES OF COLLEGE WOMEN AS INDICATED BY NITROGEN, CALCIUM AND PHOSPHORUS OUTPUTS[1]

M. M. KRAMER, H. F. EVERS, M. G. FLETCHER AND D. I. GALLEMORE
Kansas Agricultural Experiment Station, Manhattan

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Accepted figures are available for the amounts of protein and minerals thought desirable in the human diet, and various methods are used to learn how actual dietaries compare with these standards. Group dietary studies yield valuable information, but do not show the actual intake of each subject. On the other hand, accurate individual weighed dietary studies can seldom be conducted without interfering in some way with free choice of food by the subjects. Another method, which can be used for adult subjects eating their usual freely chosen diets, is the determination of output as an indication of dietary intake. Unless the intake is at a very low level, the normal adult tends to adjust nitrogen and mineral metabolism to his supply, so that determinations of the amounts eliminated indicate the amounts contained in the diet. For the work here described, the protein, calcium and phosphorus intakes of twenty-five college women were studied in this manner.

Healthy college women, graduate and undergraduate students, served as subjects for 4 days in the fall and again for 4 days in the winter, each making complete collections of urine and feces. Subjects were weighed daily during the experimental periods, and the average weights for each subject for both periods (table 1) were available for use in making

[1] Contribution No. 17 Department of Home Economics.

calculations. The subjects were asked to follow their customary habits, including between-meal eating. In most cases they were not informed as to the exact nature of the experiments so that spontaneous choice of food might not be affected.

TABLE 1

Weights of subjects used

	WEIGHT IN KILOGRAMS	
	Fall period	Winter period
A	49.0	49.9
B	73.5	73.9
C	56.2	55.8
D	54.4	56.2
E	49.9	50.8
F	58.1	59.0
G	52.2	54.9
H	55.3	56.2
I	57.6	59.4
J	70.3	68.5
K	51.7	53.0
L	56.7	58.5
M	57.2	59.0
N	57.6	63.0
O	56.7	58.1
P	56.2	57.2
Q	58.1	59.4
R	48.1	55.8
S	58.5	58.5
T	42.6	43.1
U	60.3	62.1
V	55.3	55.8
W	51.7	51.7
X	54.0	54.0
Y	62.1	64.4
Average	56.1	57.5

Full lists of foods eaten were kept by the subjects. In no case did a period include a holiday or special occasion when meals would likely vary from the normal and every effort was made to secure samples at a time when students were eating in their customary manner.

The urine for each day was measured and samples preserved and the feces for each period were dried and prepared for analysis in the usual manner. Nitrogen determinations were made on daily samples of urine for all subjects, using the Kjeldahl quantitative method. Quantitative determinations on urine and feces for each period for each subject were made for calcium according to the volumetric method of McCrudden ('11) and for phosphorus according to the volumetric method of the Association of Official Agricultural Chemists ('25). All determinations were made in duplicate.

The amount of average daily urinary nitrogen was calculated for each subject for both periods. Protein ($N \times 6.25$) used per day was calculated, using the commonly accepted assumption that approximately 10 per cent of the food protein is lost in the feces. Average daily calcium and phosphorus outputs were calculated for each subject for each period from determinations on urine and feces. These figures, to be used as indicative of nitrogen (or protein), calcium and phosphorus intakes, were calculated to the common basis of 70 kg. of body weight so that comparisons could be made.

Chittenden ('32) summarizing from his extensive investigations concerning the minimum protein requirements of the human body, concludes "that man may apparently adjust himself to a lowered intake of protein food, with a protein metabolism corresponding to 0.12 gram of nitrogen per kilogram of body-weight." This corresponds to 53 gm. of protein per 70 kg. per day. Using the best data now available, Sherman ('32) finds the average for normal adult maintenance to be about 0.5 gm. protein per kilogram of body weight per day. While the Sherman minimum requirement is, on this basis, 35 gm. of protein per 70 kg. per day; the suggested standard for adults, allowing a desirable margin for safety, is 1 gm. of protein per kilogram of body weight or 70 gm. of protein per 70 kg. per day. Similarly, data calculated to a uniform basis of 70 kg. of body weight have given the Sherman ('20 b) calcium requirements of 0.45 gm. per day and the Sherman ('20 a) phosphorus requirement of 0.88 gm. per

day. Sherman dietary standards ('32) per 70 kg. per day are 50 per cent above the minimum requirements, that is, 0.68 gm. calcium and 1.32 gm. phosphorus. The data secured from the twenty-five subjects, eating their customary diets, have been arranged in table 2, so that results from various subjects may be compared with each other and also with accepted standards for the adult human diet, the average daily figures for protein, calcium and phosphorus used per day, as calculated from output, being taken as indicative of the dietary intake.

Results for protein are distinctly different from those for calcium and phosphorus. Values equal to or above the Sherman standard of 1 gm. of protein per kilogram of body weight were found for only seven subjects in the fall and for three subjects in the winter. While no subject showed amounts below the Sherman requirements, six in the fall and four in the winter were below the Chittenden minimum. A different distribution was found for the figures for calcium and also for phosphorus, in every case a majority being above the Sherman standard. It is interesting to note that the calcium figures showed that more than half the subjects were using at least 1 gm. of calcium per day, the amount advocated for children by Sherman and Hawley ('22). In the whole series, one subject only showed a figure below the Sherman requirements, that being for calcium in the fall period.

While the figures for the Sherman standards and the Sherman requirements for calcium and phosphorus were calculated on the basis of 70 kg. of body weight, they are sometimes used as amounts per person per day. This is particularly true in discussions of dietary practices for women, since it is felt that the female should be provided with a generous rather than a scanty supply of calcium and phosphorus. Therefore, counts were made to determine the distribution of subjects on the basis of protein, calcium and phosphorus used per subject per day. This distribution shown as percentage of the number of subjects, is compared in table 3 with the distribution when the count is made on the basis of 70 kg. of body

TABLE 2

Distribution of subjects according to average amounts of protein, calcium and phosphorus used per day calculated to uniform basis of 70 kg. of body weight

FALL PERIOD						WINTER PERIOD					
Protein		Calcium		Phosphorus		Protein		Calcium		Phosphorus	
	Grams daily		Grams daily		Grams daily		Grams daily		Grams daily		Grams daily
		R	1.84					F	1.78		
		N	1.59					E	1.71		
		D	1.49					R	1.52		
		F	1.37					H	1.49		
		O	1.25					T	1.47	E	2.14
		I	1.20					W	1.42	T	1.67
		M	1.18					B	1.41	F	1.66
		Y	1.18					O	1.33	O	1.63
		K	1.14	D	1.96			K	1.32	I	1.53
		X	1.12	X	1.78			C	1.21	X	1.49
		W	1.10	E	1.77			M	1.07	H	1.48
		E	1.07	R	1.75			L	1.06	R	1.44
		A	1.06	L	1.68			I	1.05	B	1.42
		P	0.97	I	1.58			N	1.05	C	1.42
		G	0.93	O	1.58			X	1.02	M	1.42
E	99.0	V	0.93	K	1.49			D	1.01	K	1.41
L	77.9	U	0.89	Y	1.49			Q	1.00	Q	1.39
Y	77.5	Q	0.87	A	1.43			G	0.94	U	1.38
T	75.8	L	0.85	N	1.43			P	0.87	W	1.37
X	74.9	H	0.84	G	1.39	T	90.8	A	0.78	G	1.36
I	74.4	B	0.74	W	1.35	E	84.3	J	0.77	L	1.36
S	70.7	C	0.70	V	1.34	I	78.8	U	0.69	D	1.33
Sherman standards, per 70 kg. of body weight											
	70.0		0.68		1.32		70.0		0.68		1.32
K	69.5	T	0.66	T	1.28	G	68.6	V	0.67	A	1.29
P	66.8	J	0.54	H	1.27	R	65.9	Y	0.63	J	1.22
Q	63.1			P	1.27	Q	64.7	S	0.50	P	1.21
A	62.9			B	1.25	P	62.1			Y	1.21
R	62.4			J	1.23	K	61.7			N	1.16
H	60.3			M	1.22	M	61.1			V	1.08
V	58.8			U	1.16	L	60.9			S	0.97
W	58.8			Q	1.14	A	60.5				
D	58.7			F	1.04	H	59.3				
U	58.7			S	1.02	Y	58.7				
O	58.6			C	0.96	X	57.8				
G	57.8					D	57.6				
						N	56.6				
						F	55.6				
						V	55.6				
						U	54.9				
						W	54.4				
						O	52.5				
	...[1]					..	...[1]				
M	51.0					B	51.6				
J	50.8					C	49.4				
B	47.8					S	47.0				
F	47.6					J	46.2				
C	46.0										
N	39.9										
Sherman requirements, per 70 kg. of body weight											
	35.0		0.45		0.88		35.0		0.45		0.88
		S	0.44								
Aver.	62.8		1.04		1.39		60.7		1.11		1.40

[1] Chittenden minimum, 0.12 gm. of nitrogen per kilogram of body weight.

weight per day. By either method of counting, figures for many subjects are below the desired standards. Although values for no subjects fall below the protein requirement, the fact that more than two-thirds of them are below the protein standard may be a matter of some concern. While a good majority of the subjects have figures above the calcium and phosphorus standards per 70 kg. of body weight per day, it should be noted that some are below the requirements, particularly when considered per subject per day.

TABLE 3

Percentage distribution of subjects according to amounts of protein, calcium and phosphorus used

PER CENT OF SUBJECTS	PER SUBJECT PER DAY						PER 70 KG. PER DAY					
	Fall			Winter			Fall			Winter		
	Protein	Ca	P	Protein	Ca	P	Protein	Ca	P	Protein	Ca	P
Above Sherman standards[1]	28	84	12	12	80	20	28	88	56	12	88	72
Below Sherman standards, but above Sherman requirements	72	8	72	88	16	72	72	8	44	88	12	28
Below Sherman requirements[2]	00	8	16	00	4	8	00	4	00	00	00	00

[1] One gm. of protein per kilogram of body weight per day, 0.68 gm. calcium, 1.32 gm. phosphorus.

[2] One-half gm. protein per kilogram of body weight per day, 0.45 gm. calcium, 0.88 gm. phosphorus.

Relationships between the figures have been studied. In general, it seems that a subject showing low figures in one place is likely to have low figures elsewhere and a subject listed at the top of one column is apt to be high in others. Subjects having adequate supplies of calcium are likely to have good amounts of phosphorus, and vice versa, the coefficients of correlation between the amounts of calcium and phosphorus being 0.94 (a nearly perfect correlation) for the fall period and 0.78 (a good correlation) for the winter period. Some direct correlation also exists between amounts of protein and of phosphorus, the coefficients of correlation being 0.55 for the fall period and 0.61 for the winter period. No direct

correlation should be demonstrated between protein and calcium used.

Figures for each subject were used to study differences between the two periods. Of the twenty-five subjects, ten had more protein per 70 kg. in the fall, fourteen had more calcium and eleven had more phosphorus. Averages for protein, calcium and phosphorus per 70 kg. for the twenty-five subjects for the fall period are similar to those for the winter period. Whether studied subject by subject or on the basis of averages, no significant differences were found between the fall and winter periods.

Daily food lists which had been kept by the subjects were checked in an effort to find relationships between foods consumed and the protein, calcium and phosphorus used. These lists proved less helpful than anticipated, no doubt on account of variation in estimation of amounts. Counts of high calcium and phosphorus foods, especially milk and cheese, were found to correspond fairly well with the calcium and phosphorus used. The liberal use of milk, with correspondingly generous supplies of calcium and phosphorus, may perhaps be accounted for by the fact that all the subjects had had college courses in foods and nutrition and had learned of the value of milk in the diet.

In the original selection of subjects for this study, an effort was made to have some who ate freely chosen meals, as in cafeterias, and some who ate set meals, as in the college dormitory under college supervision or in organized houses. When the data were studied, only negligible differences were found between the average amounts of protein, calcium and phosphorus used per 70 kg. per day by the group eating freely chosen meals and the group eating set meals.

SUMMARY

Nitrogen, calcium and phosphorus outputs of twenty-five college women, eating freely chosen diets, have been determined to indicate protein, calcium and phosphorus intakes, using two periods of 4 days each.

In no case were daily protein figures below the Sherman requirement, but a majority were below the Sherman standard of 70 gm. of protein per 70 kg. of body weight. Protein per 70 kg. per day averaged 62.8 gm. for the fall period and 60.7 gm. for the winter period.

Calcium per 70 kg. per day averaged well above the Sherman standard of 0.68 gm., namely, 1.04 and 1.11 gm. for the fall and winter periods, respectively. Similarly, phosphorus per 70 kg. per day averaged 1.39 and 1.40 gm. for the two periods, both figures being above the Sherman standard of 1.32 gm.

Figures for protein used by the twenty-five subjects give averages distinctly below accepted standards, suggesting that the majority of the subjects were consuming less protein than is often advised. While averages for calcium and phosphorus were above the accepted standards, figures for some subjects were below accepted standards, in one case the calcium figure falling below the calcium requirement. Because some of the subjects were below the desired standards, it would seem a matter of concern to include sufficient high calcium and high phosphorus foods in the diet.

Whether studied subject by subject or on the basis of averages, no significant seasonal variations were found.

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FURTHER EXPERIMENTS WITH CATARACT IN ALBINO RATS RESULTING FROM THE WITHDRAWAL OF VITAMIN G (B_2) FROM THE DIET

PAUL L. DAY AND WILLIAM C. LANGSTON
Departments of Physiological Chemistry and Anatomy, School of Medicine, University of Arkansas, Little Rock

TWO FIGURES

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In a former paper (Day, Langston and O'Brien, '31) the authors described the occurrence of cataract in albino rats receiving a diet deficient in vitamin G (B_2). In a series of thirty-seven rats receiving the deficient diet, thirty-five developed cataract, the average time of appearance being the eighty-second day on the diet. Since that report, additional cases of cataract have developed in animals receiving the deficient diet. Cataracts have also been developed in animals under three other dietary regimes, in which the vitamin G was completely or only partially withheld. This lesion has appeared with such regularity that it can hardly be an isolated or accidental phenomenon. Not only the regularity of appearance, but also the specific nature of the lesion recommends it as a criterion of the vitamin deficiency. A dermatitis has been observed by all investigators working with vitamin G (B_2), but the manifestations of the condition vary widely. The dermatitis may appear as a loss of hair, as a roughening of the fur, as a mild erythema, or as distinct scabby ulcers on the face, back of the head, body, or legs. How much of the alopecia and ulceration is due to scratching is a matter of question. A stomatitis and salivation are frequently observed, but are by no means constant symptoms. On the other hand, the appearance of cataract has been found to be unmistakable and quite uniform. With very little experience it

can be observed with the naked eye in albino rats. With the ophthalmoscope it can, of course, be observed earlier and with greater precision. Since the preparation of this manuscript for publication, the authors' earlier work showing that cataract results from G-avitaminosis has been confirmed by O'Brien ('32) and Yudkin ('33).

EXPERIMENTAL

The animals used were of known dietary history, coming from stock which had received Sherman's diet 13 ('24) ad libitum and frequent feedings of fresh meat and lettuce for several generations. The young were separated from their mothers at about 21 days of age, or when they weighed 35 to 45 gm. To prevent coprophagy, they were kept in metal cages with raised floors of $\frac{3}{8}$ inch hardware cloth. The diets used were in all cases modifications of the Sherman and Spohn vitamin B complex deficient diet ('23), differing from each other chiefly in the manner in which the antineuritic vitamin was supplied. Diet 554 is the diet used by Bourquin and Sherman ('31) in their technic for the determination of vitamin G, the vitamin B (B_1) being supplied by an 80 per cent alcoholic extract of ground whole wheat. Diet 625 differs from it only in that the vitamin B is supplied by an 80 per cent alcoholic extract of rice polish. This extract is prepared as follows: 500 gm. of rice polish are stirred with 1500 cc. of 80 per cent (by weight) alcohol for a half hour or longer, allowed to stand overnight, again stirred for a half hour and filtered on a Büchner funnel. The rice polish is washed with 500 cc. of the 80 per cent alcohol in several small successive portions, and the filtrate and washings combined and evaporated at room temperature in front of an electric fan. This extract is finally evaporated down on cornstarch and the starch so prepared incorporated in the diet.

In diet 615, the vitamin B is supplied by the rice polish extract, but 40 per cent of the cornstarch is replaced by sucrose. This diet was used because Leader ('30) found that the presence of cane sugar in the diet increased the suscepti-

bility of rats to dermatitis. Diet 635 contains 25 per cent ground whole wheat, which supplies the necessary vitamin B, but also contains a limited quantity of vitamin G. This diet is essentially the same as that used by Sherman and Axtmayer ('27), Hunt ('28), and others. The exact compositions of these diets are summarized in table 1.

Diets 554, 615, and 625 are all deficient in, and possibly entirely free of, vitamin G. Young rats given these diets gained in weight rather rapidly for the first 2 weeks, more slowly

TABLE 1

Composition of vitamin G-deficient diets

	BOURQUIN-SHERMAN DIET 554	DIET 615	DIET 625	DIET 635
	Per cent	*Per cent*	*Per cent*	*Per cent*
Casein [1]	18	18	18	18
Salt mixture [2]	4	4	4	4
Butter fat	8	8	8	8
Cod liver oil	2	2	2	2
Cornstarch	68	28	68	43
Sucrose	..	40	..	..
Whole wheat	..	..	..	25
80 per cent alcoholic extract of whole wheat, equivalent to	50	..	..	..
80 per cent alcoholic extract of rice polish, equivalent to	..	25	25	..

[1] Extracted with 60 per cent alcohol according to the method of Sherman and Spohn ('23).

[2] Osborne and Mendel salt mixture ('19).

for the next 2 or 3 weeks, remained nearly constant in weight for some time, then gradually declined in weight until death; the weight at death usually being greater than the initial weight (fig. 1). The first symptom to appear was usually alopecia, the hair frequently starting to come out during the second or third week and often leaving the animal quite denuded. Some litters showed no loss of hair at all, however. Later, scabby ulcers sometimes appeared on various parts of the body, and salivation and stomatitis were frequently observed. During the seventh or eighth week a conjunctivitis

and keratitis, with or without corneal vascularity, accompanied by a loss of hair around the eyes, were usually seen. These signs were followed in a short time by a dullness of the eyeball which later developed into an unmistakable opacity (fig. 2).

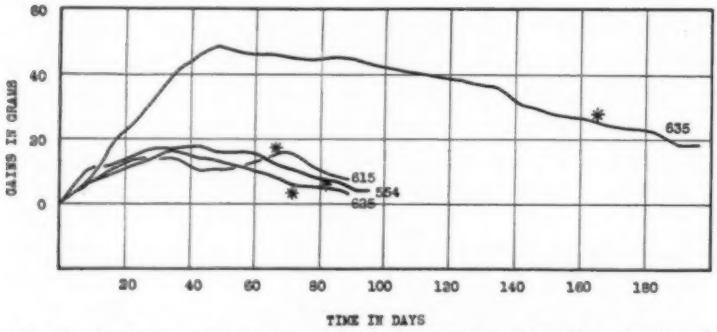

Fig. 1 Average growth curves of animals receiving four diets deficient in vitamin G (B_2). The diet number is indicated at the end of each curve, and the asterisk marks the average time of appearance of cataract.

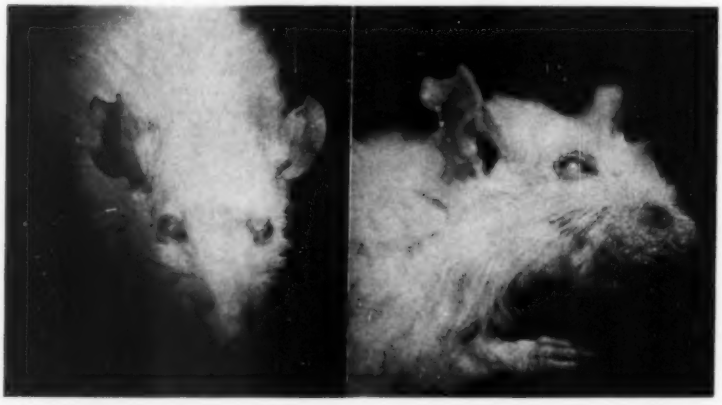

Fig. 2 Photographs showing bilateral cataract resulting from a deficiency of vitamin G (B_2). This rat developed unmistakable cataract on the fifty-second day on diet 615, and was photographed on the ninety-third day. In addition to cataract, this animal showed a conjunctivitis and keratitis, stomatitis, salivation, epistaxis, and a dermatitis with ulceration on the abdomen, but no extensive alopecia.

As diet 635 contains a small amount of vitamin G, growth was more prolonged, the maximum increment in weight correspondingly greater, and the average survival period longer. These data are represented graphically in the figure, and are also given in table 2 together with other significant data.

TABLE 2

Summary of data concerning the incidence of cataract, weight increase, and survival period of albino rats receiving four diets deficient in vitamin G

DIET NO.	NUMBER OF RATS	CATARACT			AVERAGE MAXIMUM INCREASE IN WEIGHT		AVERAGE SURVIVAL
		Number	Incidence	Average time of appearance			
			Per cent	*Days*	*Grams*	*Days*	*Days*
554	2	2	100	82	18	42	96
615	4	4	100	67	16	49	...
625	72	70	97	72	16	35	86
635	5	5	100	165	50	71	198

DISCUSSION

Of the seventy-two rats receiving diet 625, seventy developed cataract between the fortieth and the eighty-seventh day on the diet, the average time of appearance being the seventy-second day. The incidence was thus 97 per cent. In compiling the data, only those animals that lived until the minimum time of appearance of cataract were included; those animals which died or were killed before that time were arbitrarily excluded from the tabulation. For this diet the average maximum increase in weight was 16 gm., and the average time required for the rats to attain the maximum weight was 35 days. The average survival period for rats in this group was 86 days, this figure representing the average of somewhat fewer than seventy-two animals, as many were chloroformed for histological section or for other reasons and were therefore not included in the calculations.

Both of the animals given the Bourquin and Sherman diet 554 developed cataract, as did all four of the rats given the diet containing 40 per cent sucrose (diet 615), the incidence of

cataract for these diets being 100 per cent, therefore. The time of appearance of cataract, the rate of growth, and the survival period were essentially the same for these groups as for the animals receiving diet 625. As they appeared to respond in every way exactly like the animals receiving diet 625, there seemed no necessity for increasing the number of cases.

With diet 635 growth was more rapid and continued longer, and the survival period was correspondingly greater, as would be expected because of the presence of vitamin G in the ground whole wheat present in the diet. Also, the cataracts appeared later and at a similar point on the growth curve, as is evident from an examination of the chart where the average time for the appearance of cataract is indicated by the asterisk. It appears that the factor responsible for the greater growth and longer survival in diet 635 also delayed the onset of the lens opacity. In fact, the ratio of days of growth to days for appearance of cataract is approximately the same for diets 625 and 635, although the absolute number of days of growth and days for appearance of cataract differ greatly. Similarly, the ratio of days survival to days for appearance of cataract is almost exactly the same for the two diets. That is, the growth factor and the cataract-preventive factor appear to be the same, or if not the same are present in like physiological amounts. It is significant that all of the five animals receiving diet 635 developed cataract. The presence of a small amount of a hypothetical cataract-preventive factor delayed for a time the appearance of the lesion, but was not able to prevent its ultimate development.

Diagnosis of cataract was made in every case by simple gross observations with the naked eye. Dilatation of the pupil was not necessary in the albino rats, because of the absence of iris pigmentation. This diagnosis of cataract was confirmed by ophthalmoscopic observations and histological section in enough cases to dispel any doubt as to the accuracy of diagnosis. Routine examinations with the ophthalmoscope were not made in all cases in the series reported in this paper. Had such observations been made, the time reported for the

appearance of cataract would have been materially shortened, as early changes in the lens can be observed with the ophthalmoscope, while the cataract must be rather far advanced to be evident in gross observation. Also, it is possible that the two animals which received diet 625 and are reported as not developing cataract, might have had early changes which would have been discernible with the ophthalmoscope, but which were not seen without the use of such an instrument. These two animals devoured the viscera of cage mates which died of the deficiency, and therefore obtained enough vitamin G to delay the onset of cataract.

Ample evidence has been accumulated in this laboratory and elsewhere to indicate that the diets used in these experiments contain an abundance of the antineuritic vitamin, or at least an adequate amount for much greater growth than was obtained. As the development of cataract was prevented in numerous cases by the feeding of autoclaved yeast, it appears that the cataract-preventive factor is a heat-stable vitamin. Whether it is identical with the dermatitis-preventive factor (P-P, vitamin G or B_2), or whether it is one of the other factors present in the vitamin B complex is a question that cannot be answered at the present time. Inasmuch as the lens of the eye has its embryological origin as an invagination of the body wall ectoderm and therefore comes from the same germ layer as the skin, it is not unreasonable to believe that a deficiency which results in pathological changes in the skin might also result in pathological changes in the lens of the eye, even though these tissues are apparently so unlike.

The accumulated evidence for the existence of factors in addition to the six commonly recognized vitamins has been largely based on growth (Harris, '32). Undoubtedly the new factors reported from some laboratories are identical with those reported from others, but because of the different methods of experimentation used it is difficult to correlate this mass of evidence. As growth is not specific, it would seem to the authors that one of the most useful methods in clarifying the situation would be to establish a definite pathology

with each deficiency, wherever this is possible. This pathological condition could then he recognized by other investigators, and the various lines of work thus correlated. The polyneuritis of vitamin B (B_1) deficiency is easily recognized, the dermatitis of vitamin G (B_2) deficiency has been quite commonly observed, and the symptoms accompanying a deficiency of Reader's vitamin B_4 have been described, but the pathological conditions resulting from deficiencies of the other postulated factors are less well known. It is possible that cataract is a manifestation of lack of one of these new factors, or, as seems more probable now, it is another manifestation of vitamin G (B_2) deficiency. If the latter is true, we have another and perhaps better criterion of vitamin G deficiency. The term 'dermatitis' is in itself indefinite, and as was pointed out in the introduction, the lesions produced by withdrawal of vitamin G are not specific and vary widely in different animals. Even with histo-pathological sections of the skin it is difficult to establish any very characteristic pathology as a result of the deficiency. The crystalline lens of the eye, on the other hand, is a transparent tissue that can be readily observed without injury to the animal, and in which even early changes are definite and unmistakable.

Salmon, Hays, and Guerrant ('28) showed that the presence of certain bacteria is an etiological factor in the development of the ulceration of vitamin G dermatitis. It is probable that scratching and other external irritations also contribute to the ulceration and alopecia. With these factors contributing to the development of the dermatitis, it is not surprising that there is considerable variation in the appearance of the signs of the deficiency. The position of the lens of the eye, however, would operate against its being affected by such external influences in experimental animals. Bacterial invasion could hardly be a factor in the development of the cataract; since the lens of the eye is non-vascular, even blood-borne organisms could not affect it directly. With a systemic disturbance as the only probable cause of the cataract it would appear that, theoretically at least, cataract should be a more reliable criterion of the deficiency than dermatitis.

SUMMARY

Cataracts have developed in nearly 100 per cent of the albino rats given diets freed of vitamin G (B_2). Cataracts also developed in all the animals given a diet containing 25 per cent whole wheat and therefore containing a limited quantity of vitamin G. With this latter diet, however, the cataracts appeared at a later date, and the time of their appearance can be correlated with the greater growth and longer survival of this group of animals. It thus appears that in the diets used the growth-limiting and cataract-preventive factors are identical, or else these diets are similarly deficient in both.

Cataract is suggested as a better criterion of vitamin G deficiency than dermatitis, as its appearance is more consistent and unmistakable, and also as its development is not influenced by external irritations and bacterial invasion which are undoubtedly contributing factors in the development of dermatitis.

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RELATION BETWEEN DEHYDRATION AND THE INSENSIBLE LOSS OF WATER[1]

L. H. NEWBURGH AND MARGARET WOODWELL JOHNSTON
Department of Internal Medicine, Medical School, University of Michigan

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In earlier papers we presented evidence to support our conviction that the heat production for periods of 24 hours or longer may be satisfactorily calculated from a knowledge of the amount of water vaporizing during the period. At that time, various sources of error were studied and discussed. We neglected, however, to deal experimentally with possible shifts in the insensible water due to differences in the water content of the organism.

It was already known that extreme depletion of the body's water would diminish the vaporization of water from the skin and lungs. Under these unusual circumstances, the method would no longer be valid. On the other hand, it was assumed that the moderate increases or decreases in the organism's content of water, such as might be commonly encountered, would not change the rate at which heat was being removed by vaporization of water.

But a group of investigators (Manchester, Husted and McQuarrie, '31) have recently come to the conclusion that changes in the degree of hydration are a much more important source of error than we had supposed. We have tried to emphasize in our earlier papers (Newburgh, Wiley and Lashmet, '31) the importance of calculating the heat production from the insensible loss of water, not from the insensible loss

[1] The expenses of this investigation were defrayed, in part, by a fund for the study of nutrition, created by Mr. W. K. Kellogg, of the Kellogg Corn Flake Company, Battle Creek, Michigan.

of weight, since the vaporized water may be as little as 82 per cent, or as much as 104 per cent of the insensible loss of weight, depending on the nature and amounts of the materials oxidized. The investigators just referred to did not, however, make the comparison in this way, even though the metabolic mixture of a single subject changed greatly from the foreperiod to that of dehydration. They were of necessity, then, dealing with at least two variables and totally ignoring one of them. We accordingly felt it necessary to investigate this question.

The subject was an apparently normal, highly educated young man (age 23 years, weight 60 kg., height 180 cm.), who understood the nature of the problem and who honestly tried to cooperate with us in every way. Two groups of experiments were carried out. The first group consisted of five consecutive periods. Throughout these studies, that occupied about 6 months, the subject lived in the hospital room especially designed for the quantitative study of metabolic problems. He was not confined to bed, but occupied himself for the most part by studying and writing. He was permitted to leave his room to go to the shower and telephone, and selected visitors were allowed. He played cards at times with one of the internes. The range of his activity is manifested by the variation in his heat production (calculated from his insensible loss of water) during the 53 days when his water intake was inadequate. His outgoing Calories varied from 2330 to 3008. All the weighings and the preparation of all the food were carried out by one of us.

The subject was fed a uniform diet selected for its dryness. In each period, he first received an adequate supply of drinking water and then various degrees of dehydration were produced by limiting this supply. The weight of the insensible water was determined by the method fully described by us (Newburgh, Wiley and Lashmet, '31). In general, less water was vaporized from the lungs and skin during the periods of dehydration than during the control periods. There was, however, no constant relationship between the degree of dehydra-

tion and the fall in the insensible loss of water. It may be seen from table 1 that the largest dehydration was accompanied by the largest insensible water.

TABLE 1

Summary of preliminary experiments

EXP. NO.	NUMBER DAYS	BODY WATER LOST	AVERAGE INSENSIBLE WATER	MINIMAL INSENSIBLE WATER
		Gm.	*Gm.*	*Gm.*
2 (a)	3	769	903	886
5	3	1525	874	796
4	4	1888	889	836
2 (b)	9	2267	844	718
1	4	2624	749	661
3	4	3227	985	935

Further, it was evident to both the subject and to us that the discomfort produced by the curtailment of water led him to omit many of his accustomed activities. For example, we noted that he was lying on his bed the greater part of the day. This was especially true in the first experiment. He gave up writing entirely during these periods. The average insensible loss of water—827 gm.—during 39 days of dehydration as opposed to 1082 gm., the average for 53 control days, may easily be accounted for by lessened activity. If the percentage of heat lost by the vaporization of water remained the same, such a decrease would represent a fall in heat production from about 2600 Calories to about 2000 Calories.

With these considerations in mind, we repeated the experiment under somewhat different conditions. The same subject was again used. This time he occupied a quiet room in the laboratory away from all the noises and confusion of the ordinary hospital ward. He himself reported that he was much less restless and not disturbed by distractions under these new conditions. He was at times visited by his wife and young baby. Throughout the experiment he was fed the same diet every day. Its composition was as follows: protein (by analysis) 54 gm.; fat 147 gm.; carbohydrate 232 gm., that

yielded 2484 Calories.[2] The dry solids of this diet by actual determinations were 435 gm. Its water content varied from 392 to 426 gm.

The experiment extended over 18 days and consisted of four consecutive periods: 8 days, foreperiod; 4 days, dehydration; 3 days, recovery; 3 days, dehydration. During the foreperiod and the first dehydration, no salt was added to the diet. Its low ash content is indicated by the low chloride excretion of the foreperiod, which averaged 0.55 gm. Cl. daily. In the recovery period and the second dehydration 10 gm. of sodium chloride were ingested daily.[3] This addition was made in order to obtain a greater depletion of body water. During the foreperiod the subject drank approximately 1250 gm. of water per day. Dehydration was effected in both cases by omitting all drinking water. The body water lost during the first period of dehydration was restored in the first day of recovery by allowing about 3000 gm. of drinking water. During the second and third days of recovery, the subject again received about 1250 gm. of drinking water daily.

The pertinent data are given in table 2. From this we have constructed table 3, which shows the relationship of the insensible loss of water to changes in body water. It will be seen that under these new conditions the subject was less active than in the earlier studies, and that the range of his activity was also less. It may further be noted that his activity decreased as he became adjusted to his hospital routine. The average 24 hourly insensible loss of water for the first 4 days was 835 gm., as compared with 766 gm. for the second 4 days. The average insensible loss of water for each of the subsequent three periods is as follows: First dehydration 782 gm.; recovery 771; second dehydration 748

[2] The actual foods fed were: Puffed rice 12 gm.; Uneeda biscuits 70 gm.; butter (salt-free) 60 gm.; cream (40 per cent) 180 gm.; sugar 35 gm.; 'mints' 75 gm.; jelly 20 gm.; potato 150 gm.; canned peas drained 120 gm.; lettuce 50 gm.; walnut meats 20 gm.; lean beef 135 gm. To obtain a uniform preparation sufficient meat for the period was ground after being freed of its fat, and preserved by freezing.

[3] The sodium chloride was administered in gelatin capsules which contained 0.25 gm. nitrogen.

gm. If one compares these values with the average for the second 4 days of the control period, it is obvious that no significant differences exist.

Since the subject pointed out that he was disinclined to exert himself during the periods of dehydration, a comparison may be made between quiet days of the foreperiod and those

TABLE 2

Data

DATE	AVAILABLE WATER			EXCRETED WATER		INSENSIBLE LOSS OF WEIGHT	
	Ingested	Preformed	By oxidation	Urine and stool	Insensible		
1932 Oct.							
13	1677	—6	255	816	844	935	Diet: Protein 54; fat
14	1647	—6	255	1157	895	987	147; carbohydrate 232.
15	1617	—6	255	1117	840	932	Metabolic mixture: Pro-
16	1674	—6	255	872	763	855	tein 54; fat 83; carbo-
17	1674	—6	255	1339	763	855	hydrate 232.
18	1662	—6	255	1144	708	800	$CO_2 - O_2 = 92$
19	1654	—6	255	1144	754	846	
20	1675	—6	255	1266	840	932	
21	408	—6	250	380	887	980	Diet: Same.
22	394	—6	250	374	765	858	Metabolic mixture: Pro-
23	426	—6	250	581	735	828	tein 54; fat 83; carbo-
24	410	—6	250	340	740	833	hydrate 232. $CO_2 - O_2 = 93$
25	3434	+8	243	580	735	829	Diet: Same + 10 gm.
26	1677	+8	243	861	787	881	NaCl + 1.3 gm. gelatin.
27	1702	+8	243	1441	790	884	Metabolic mixture: Pro-
28	392	+8	243	1027	768	862	tein 60.4; fat 74; carbo-
29	421	+8	243	811	723	817	hydrate 232.
30	404	+8	243	752	752	846	$CO_2 - O_2 = 94$.

of dehydration. For this purpose we selected from the control period the 4 days during which his activity was smallest. The average insensible loss of water for these days is 747 gm. With this may be compared the average of 746, for the last 3 days of the first dehydration, and 748 for the second dehydration. The uniformity of these three averages is striking.

Further, it should be pointed out that the lowest output of

insensible water (708 gm.) of the whole 18 days occurred on the sixth day of the control period, when the subject was receiving sufficient water.

TABLE 3

Relation between water content of body and insensible loss of water

DATE	BODY WATER ADDED OR LOST	INSENSIBLE WATER	TOTAL CALORIES [1]
Foreperiod. Drinking water about 1250 gm. daily. Low salt. Body water added, 150 gm.			
Oct., 1932			
13	+ 236	844	2042
14	— 156	895	2166
15	— 91	840	2033
16	+ 288	763	1846
17	— 179	763	1846
18	+ 59	708	1713
19	+ 35	754	1825
20	— 42	840	2033
First dehydration. No drinking water. Low salt. Body water lost, 2188 gm.			
21	— 615	877	2147
22	— 501	765	1851
23	— 646	735	1779
24	— 426	740	1791
Recovery. Drinking water 3000 gm. first day. Then 1250 gm. daily. High salt. Body water added, 2369 gm.			
25	+ 2370	735	1779
26	+ 280	787	1905
27	— 281	790	1912
Second dehydration. No drinking water. High salt. Body water lost, 2863 gm.			
28	— 1152	768	1859
29	— 862	723	1750
30	— 849	752	1820

[1] Calculated from the insensible water.

Finally if dehydration of the magnitude produced here disturbs the mechanism of dissipation of heat by vaporization of water, one would expect a decreasing insensible loss of water as dehydration increased. That this is not at all the case may be seen in table 2. Thus in the first dehydration

the insensible losses for the third and fourth days were respectively 735 and 740 gm. Likewise, in the second period of dehydration, the losses for the last 2 days were 723 and 752 gm.

We also produced a moderate degree of dehydration by the administration of ammonium chloride. The subject received 52 gm. in 4 days. The ingestion of water was the same as in the foreperiod. A loss of body water of only 1480 gm. resulted. The average loss of insensible water for the 4 days was 1243 gm., as compared with 1151 gm. for the control period. As a result of the ammonium chloride ingestion, the subject became restless and hyperpneaic. The acidosis, as evidenced by the hypernea, must have resulted in an increased output of CO_2. Since we were unable to measure the CO_2 output for 24 hours, we calculated the $CO_2 - O_2$ difference from the metabolic mixture in the usual way. This must have resulted in a value somewhat too low, and therefore its subtraction from the insensible loss gave a value for the insensible water which was correspondingly too high. This error together with the restlessness due to the nausea produced by the ammonium chloride may easily account for the recorded increase in insensible water. Because of these complications it is impossible to draw a sound conclusion regarding the effect of this type of dehydration on the insensible loss of water. At any rate, there is no evidence that the loss of body water effected in this way lowers the percentage of heat lost by the vaporization of water.

We have, then, obtained no support for the idea that anything less than extreme dehydration affects this percentage. Levine and Wyatt ('32) found that the basal insensible loss of weight of sixteen dehydrated infants was on the average 11 per cent below that of the same group after recovery. They have further observed that dehydrated infants studied in a respiration chamber continue to lose the normal percentage of heat by the vaporization of water, but that the average respiratory quotient is 0.76 as contrasted with the normal of 0.85. They calculate that this depression of the

quotient could account for 8 per cent of the decrease in insensible perspiration of the dehydrated infants. The remaining 3 per cent is so small a difference that it may be considered within the limits of error of the method employed. These results dealing with the basal state appear to be in agreement with those obtained by us for the 24-hourly cycle.

As mentioned earlier, our results are not in agreement with those obtained by Manchester, Husted and McQuarrie ('31). It is unfortunate that these authors did not deal with the insensible loss of water rather than the insensible perspiration, since they fed such a variety of diets. Careful examination of their charts reveals that the insensible loss sometimes rises and sometimes falls during dehydration. In the case of W.J., on the eighth day, water was added to the body, and on the ninth day body water was lost. Nevertheless, the insensible loss was much higher on the ninth than the eighth day. Another example may be seen in the case of J.R. On the twenty-first, twenty-second and twenty-third days there was progressive marked dehydration. Nevertheless, the insensible loss was highest on the twenty-third and least on the twenty-first.

The question of activity is not dealt with. Since our subject in the first series of experiments reduced his activity greatly when deprived of water, one is led to wonder if the children used in the investigation did not vary their activity sufficiently to account for some of the irregularities in insensible perspiration.

These investigators lay great emphasis on a comparison of heat production for 12-hour periods, measured by indirect calorimetry, with the predicted heat production estimated from the insensible perspiration by means of the Benedict and Root prediction table. In this way they obtain good agreement in their control periods, while in dehydration the heat production predicted from the insensible perspiration falls too low (table 1, Manchester, Husted and McQuarrie, '31).

In the first place, it is doubtful whether the total heat for a period of 12 hours can be calculated from a series of determinations occupying only a small fraction of every 2 hours. In the second place, we have previously pointed out that the calculation of heat production from the insensible perspiration may give highly erroneous results because of the effect of the respiratory quotients. Table 4 represents a recalculation of some of their data. The insensible water was obtained from the insensible perspiration by calculation of the $CO_2 - O_2$ difference of the diets used. It will be seen that in the

TABLE 4

Recalculation of table 1 (Manchester, Husted and McQuarrie

INSENSIBLE PERSPIRATION	TOTAL CALORIES (12 HOURS)			CO_2-O_2 CALCULATED FROM DIET	DEHYDRATION
	Predicted from insensible perspiration	Predicted from insensible water	Determined (Tissot)		
Gm.				*Gm.*	*Gm.*
326	657	765	659	— 10	0
266	580	620	639	— 10	— 914
319	650	748	630	— 10	+ 259
322	654	799	672	+ 8	0
251	560	627	666	+ 8	— 1308
293	614	682	693	— 11	0
303	630	707	694	— 11	+ 300
205	500	469	664	— 11	— 1233
220	518	506	655	— 11	— 2087
212	508	486	639	— 11	— 2752

case of the first control period, line 1 of the table, the apparent agreement between the heat determined by indirect calorimetry, viz. 659 Calories, and the heat predicted from the insensible perspiration, 657 Calories, disappears when the heat is calculated from the insensible water. It then becomes 765 Calories. Since in fact wide divergence occurs in the control periods, the significance of divergences in the dehydration periods are difficult to interpret.

Further, if dehydration lowers the percentage of heat lost by the vaporization of water, one would expect dehydration of the same degree to produce the same lowering of the insensible loss of weight in the case of a single subject. That this

did not occur may be seen from table 4, where two recorded dehydrations of 1308 and 1233 gm. resulted in insensible losses of weight of 251 gm. and 205 gm. respectively. This difference becomes even greater when it is expressed as the weight of the insensible water, viz., 259 gm. and 194 gm.

Accordingly, our very careful study of this paper does not convince us that dehydration to the extent effected here influences the percentage of heat removed by the vaporization of water.

CONCLUSION

The removal of approximately 6 per cent of the body water failed to alter the percentage of heat dissipated by the vaporization of water. Dehydration of this magnitude does not, therefore, introduce an error in the calculation of the total transformation of energy from the insensible loss of water.

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